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(57) Abstract

The present invention concerns compounds and methods for modulating metabotropic glutamate receptor activity. The compounds are targeted to a binding site on the metabotropic glutamate receptor which appears to be distinct from the glutamate receptor binding site. The preferred use of the compounds and methods of the present invention is to modulate metabotropic glutamate receptor activity and thereby aid in the treatment of neurological diseases or disorders. The compounds can also be used to produce other effects such as an analgesic effect, cognition-enhancement effect, and a muscle-relaxant effect.

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DESCRIPTION

Methods And Compounds Active At Metabotropic Glutamate Receptors Useful For Treatment Of Neurological Disorders And Diseases

Field of the Invention

The present invention relates to compounds and methods for modulating metabotropic glutamate receptor activity and binding to metabotropic glutamate receptors. Modulation of metabotropic glutamate receptor activity can be used for different purposes such as treating neurological disorders and diseases, inducing an analgesic effect, cognition enhancement, and inducing a muscle-relaxant effect.

Background of the Invention

The following description provides a summary of information relevant to the present invention. It is not an admission that any of the information provided herein is prior art to the presently claimed invention, nor that any of the publications specifically or implicitly referenced are prior art to that invention.

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). Glutamate produces its effects on central neurons by binding to and thereby activating cell surface receptors. These receptors have been subdivided into two major classes, the ionotropic and metabotropic glutamate receptors, based on the structural features of the receptor proteins, the means by which the receptors transduce signals into the cell, and pharmacological profiles.

The ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that upon binding glutamate, open to allow the selective influx of certain monovalent and divalent cations, thereby depolarizing the cell membrane. In addition, certain iGluRs with relatively

high calcium permeability can activate a variety of calcium-dependent intracellular processes. These receptors are multisubunit protein complexes that may be homomeric or heteromeric in nature. The various iGluR subunits all share common structural motifs, including a relatively large amino-terminal extracellular domain (ECD), followed by a transmembrane domain (TMD) comprising two membrane spanning segments, a second smaller extracellular loop, and a third membrane spanning segment, before terminating with an intracellular carboxy-terminal domain. Historically the iGluRs were first subdivided pharmacologically into three classes based on preferential activation by the agonists alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA), kainate (KA), and N-methyl-D-aspartate (NMDA). Later, molecular cloning studies coupled with additional pharmacological studies revealed a greater diversity of iGluRs, in that multiple subtypes of AMPA, KA and NMDA receptors are expressed in the mammalian CNS (Hollman and Heinemann, Ann. Rev. Neurosci. 17:31, 1994).

The metabotropic glutamate receptors (mGluRs) are G protein-coupled receptors capable of activating a variety of intracellular second messenger systems following the binding of glutamate. Activation of mGluRs in intact mammalian neurons can elicit one or more of the following responses: activation of phospholipase C, increase in inositol phosphate, increases in phosphoinositide (PI) hydrolysis, mobilization of intracellular calcium, activation of phospholipase D, activation or inhibition of adenylate cyclase; increases or decreases in the formation of cyclic adenosine monophosphate (cAMP), activation of guanylate cyclase, increases in the formation of cyclic guanosine monophosphate (cGMP), activation of phospholipase A_2, increases in arachidonic acid release, and increases or decreases in the activity of voltage- and ligand-gated ion channels (Schoepp and Conn, *Trends Pharmacol. Sci.* 14:13, 1993; Schoepp, *Neurochem. Int.*

24:439, 1994; Pin and Duvoisin, *Neuropharmacology 34*:1, 1995).

Thus far, eight distinct mGluR subtypes have been isolated via molecular cloning, and named mGluR1 to mGluR8 according to the order in which they were discovered (Nakanishi, *Neuron 13*:1031, 1994; Pin and Duvoisin, *Neuropharmacology 34*:1, 1995; Knopfel *et al.*, *J. Med. Chem. 38*:1417, 1995). Further diversity occurs through the expression of alternatively spliced forms of certain mGluR subtypes (Pin *et al.*, *PNAS 89*:10331, 1992; Minakami *et al.*, *BBRC 199*:1136, 1994). All of the mGluRs are structurally similar, in that they are single subunit membrane proteins possessing a large amino-terminal ECD, followed by seven putative membrane spanning segments connected by three intracellular and three extracellular loops (7TMD), and terminating in an intracellular carboxy-terminal domain of variable length. While the basic topology of mGluRs is thus similar to that of all known G-protein coupled receptors, certain distinctive features are notable including the exceptionally large ECD which is 2-5 times the size of the ECD for other G-protein coupled receptors. Furthermore, mGluRs exhibit no significant amino acid sequence homology to any other G-protein coupled receptor with the single exception of the calcium receptor (Brown *et al.*, *Nature 366*:575, 1993; Nakanishi, *Neuron 13*:1031, 1994; Pin and Duvoisin, *Neuropharmacology 34*:1, 1995; Knopfel *et al.*, *J. Med. Chem. 38*:1417, 1995).

The eight mGluRs have been subdivided into three subclasses based on amino acid sequence homologies, the second messenger systems they utilize, and pharmacological characteristics (Nakanishi, *Neuron 13*:1031, 1994; Pin and Duvoisin, *Neuropharmacology 34*:1, 1995; Knopfel *et al.*, *J. Med. Chem. 38*:1417, 1995). The amino acid homology between mGluRs within a given subclass is approximately 70%, but drops to about 40% between mGluRs in different subclasses. For mGluRs in the same subclasses, this

relatedness is roughly paralleled by similarities in signal transduction mechanisms, and pharmacological characteristics.

The Group I mGluRs comprise mGluR1, mGluR5 and their alternatively spliced variants. The binding of agonists to these receptors results in the activation of phospholipase C and the subsequent mobilization of intracellular calcium. For example, *Xenopus* oocytes expressing recombinant mGluR1 receptors have been utilized to demonstrate this effect indirectly by electrophysiological means (Masu *et al.*, *Nature 349*:760, 1991; Pin *et al.*, *PNAS 89*:10331, 1992). Similar results were achieved with oocytes expressing recombinant mGluR5 receptors (Abe *et al.*, *J. Biol. Chem. 267*:13361, 1992; Minakami *et al.*, *BBRC 199*:1136, 1994; Joly *et al.*, *J. Neurosci. 15*:3970, 1995). Alternatively, agonist activation of recombinant mGluR1 receptors expressed in Chinese hamster ovary (CHO) cells stimulated PI hydrolysis, cAMP formation, and arachidonic acid release as measured by standard biochemical assays (Aramori and Nakanishi, *Neuron 8*:757, 1992). In comparison, activation of mGluR5 receptors expressed in CHO cells stimulated PI hydrolysis and subsequent intracellular calcium transients, but no stimulation of cAMP formation or arachidonic acid release was observed (Abe *et al.*, *J. Biol. Chem. 267*:13361, 1992). However, activation of mGluR5 receptors expressed in LLC-PK1 cells does result in increased cAMP formation as well as PI hydrolysis (Joly *et al.*, *J. Neurosci. 15*:3970, 1995). The agonist potency profile for Group I mGluRs is quisqualate > glutamate = ibotenate > (2S,1'S,2'S)-2-carboxycyclopropyl)glycine (L-CCG-I) > (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD). Quisqualate is relatively selective for Group I receptors, as compared to Group II and Group III mGluRs, but it also potently activates ionotropic AMPA receptors (Pin and Duvoisin, *Neuropharmacology 34*:1, Knopfel *et al.*, *J. Med. Chem. 38*:1417, 1995).

The Group II mGluRs include mGluR2 and mGluR3. Activation of these receptors as expressed in CHO cells inhibits adenlyl cyclase activity via the inhibitory G protein, G_i, in a pertussis toxin-sensitive fashion (Tanabe *et al.*, *Neuron 8*:169, 1992; Tanabe *et al.*, *J. Neurosci. 13*:1372, 1993). The agonist potency profile for Group II receptors is L-CCG-I > glutamate > ACPD > ibotenate > quisqualate. Preliminary studies suggest that L-CCG-I and (2S,1'R,2'R, 3'R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) are both relatively selective agonists for the Group II receptors (Knopfel *et al.*, *J. Med. Chem. 38*:1417, 1995).

The Group III mGluRs include mGluR4, mGluR6, mGluR7 and mGluR8. Like the Group II receptors these mGluRs are negatively coupled to adenylate cyclase to inhibit intracellular cAMP accumulation in a pertussis toxin-sensitive fashion when expressed in CHO cells (Tanabe *et al.*, *J. Neurosci. 13*:1372, 1993; Nakajima *et al.*, *J. Biol. Chem. 268*:11868, 1993; Okamoto *et al.*, *J. Biol. Chem. 269*: 1231, 1994; Duvoisin *et al.*, *J. Neurosci. 15*:3075, 1995). As a group, their agonist potency profile is (S)-2-amino-4-phosphonobutyric acid (L-AP4) > glutamate > ACPD > quisqualate, but mGluR8 may differ slightly with glutamate being more potent than L-AP4 (Knopfel *et al.*, *J. Med. Chem. 38*:1417, 1995; Duvoisin *et al.*, *J. Neurosci. 15*:3075, 1995). Both L-AP4 and (S)-serine-O-phosphate (L-SOP) are relatively selective agonists for the Group III receptors.

Finally, the eight mGluR subtypes have unique patterns of expression within the mammalian CNS that in many instances are overlapping (Masu *et al.*, *Nature 349*:760, 1991; Martin *et al.*, *Neuron 9*:259, 1992; Ohishi *et al.*, *Neurosci. 53*:1009, 1993; Tanabe *et al.*, *J. Neurosci. 13*:1372; Ohishi *et al.*, *Neuron 13*:55, 1994; Abe *et al.*, *J. Biol. Chem. 267*:13361, 1992; Nakajima *et al.*, *J. Biol. Chem. 268*:11868, 1993; Okamoto *et al.*, *J. Biol. Chem. 269*: 1231, 1994; Duvoisin *et al.*, *J. Neurosci. 15*:3075, 1995).

As a result certain neurons may express only one particular mGluR subtype, while other neurons may express multiple subtypes that may be localized to similar and/or different locations on the cell (i.e. postsynaptic dendrites and/or cell bodies versus presynaptic axon terminals). Therefore, the functional consequences of mGluR activation on a given neuron will depend on the particular mGluRs being expressed; the receptors' affinities for glutamate and the concentrations of glutamate the cell is exposed to; the signal transduction pathways activated by the receptors; and the locations of the receptors on the cell. A further level of complexity may be introduced by multiple interactions between mGluR expressing neurons in a given brain region. As a result of these complexities, and the lack of subtype-specific mGluR agonists and antagonists, the roles of particular mGluRs in physiological and pathophysiological processes affecting neuronal function are not well defined. Still, work with the available agonists and antagonists have yielded some general insights about the Group I mGluRs as compared to the Group II and Group III mGluRs.

Attempts at elucidating the physiological roles of Group I mGluRs suggest that activation of these receptors elicits neuronal excitation. Various studies have demonstrated that ACPD can produce postsynaptic excitation upon application to neurons in the hippocampus, cerebral cortex, cerebellum, and thalamus as well as other brain regions. Evidence indicates that this excitation is due to direct activation of postsynaptic mGluRs, but it has also been suggested to be mediated by activation of presynaptic mGluRs resulting in increased neurotransmitter release (Baskys, *Trends Pharmacol. Sci.* 15:92, 1992; Schoepp, *Neurochem. Int.* 24:439, 1994; Pin and Duvoisin, *Neuropharmacology* 34:1). Pharmacological experiments implicate Group I mGluRs as the mediators of this excitation. The effect of ACPD can be reproduced by low concentrations of quisqualate in the presence of iGluR

antagonists (Hu and Storm, *Brain Res.* *568*:339, 1991; Greene *et al.*, *Eur. J. Pharmacol.* *226*:279, 1992), and two phenylglycine compounds known to activate mGluR1, (S)-3-hydroxyphenylglycine ((S)-3HPG) and (S)-3,5-dihydroxyphenylglycine ((S)-DHPG), also produce the excitation (Watkins and Collingridge, *Trends Pharmacol. Sci.* *15*:333, 1994). In addition, the excitation can be blocked by (S)-4-carboxyphenylglycine ((S)-4CPG), (S)-4-carboxy-3-hydroxyphenylglycine ((S)-4C3HPG) and (+)-alpha-methyl-4-carboxyphenylglycine ((+)-MCPG), compounds known to be mGluR1 antagonists (Eaton *et al.*, *Eur. J. Pharmacol.* *244*:195,1993; Watkins and Collingridge, *Trends Pharmacol. Sci.* *15*:333, 1994).

Other studies examining the physiological roles of mGluRs indicate that activation of presynaptic mGluRs, can block both excitatory and inhibitory synaptic transmission by inhibiting neurotransmitter release (Pin and Duvoisin, *Neuropharmacology* *34*:1). Presynaptic blockade of excitatory synaptic transmission by ACPD has been observed on neurons in the visual cortex, cerebellum, hippocampus, striatum and amygdala (Pin *et al.*, *Curr. Drugs: Neurodegenerative Disorders* *1*: 111, 1993), while similar blockade of inhibitory synaptic transmission has been demonstrated in the striatum and olfactory bulb (Calabresi *et al.*, *Neurosci. Lett.* *139*:41, 1992; Hayashi *et al.*, *Nature* *366*:687, 1993). Multiple pieces of evidence suggest that Group II mGluRs mediate this presynaptic inhibition. Group II mGluRs are strongly coupled to inhibition of adenylate cyclase, like $alpha_2$-adrenergic and $5HT_{1A}$-serotonergic receptors which are known to mediate presynaptic inhibition of neurotransmitter release in other neurons. The inhibitory effects of ACPD can also be mimicked by L-CCG-I and DCG-IV, which are selective agohists at Group II mGluRs (Hayashi *et al.*, *Nature* *366*:687, 1993; Jane *et al.*, *Br. J. Pharmacol.* *112*:809, 1994). Moreover, it has been demonstrated that activation of mGluR2 can strongly inhibit presynaptic, N-

type calcium channel activity when the receptor is expressed in sympathetic neurons (Ikeda *et al.*, *Neuron*, *14*:1029, 1995), and inactivation of these channels is known to inhibit neurotransmitter release. Finally, it has been observed that L-CCG-I, at concentrations selective for Group II mGluRs, inhibits the depolarization-evoked release of 3H-aspartate from rat striatal slices (Lombardi *et al.*, *Br. J. Pharmacol.* *110*:1407, 1993). Evidence for physiological effects of Group II mGluR activation at the postsynaptic level is limited. However, one study suggests that postsynaptic actions of L-CCG-I can inhibit NMDA receptor activation in cultured mesencephalic neurons (Ambrosini *et al.*, *Mol. Pharmacol.* *47*:1057, 1995).

Physiological studies have demonstrated that L-AP4 can also inhibit excitatory synaptic transmission on a variety of CNS neurons. Included are neurons in the cortex, hippocampus, amygdala, olfactory bulb and spinal cord (Koerner and Johnson, *Excitatory Amino Acid Receptors; Design of Agonists and Antagonists* p. 308, 1992; Pin *et al.*, *Curr. Drugs: Neurodegenerative Disorders* *1*: 111, 1993). The accumulated evidence indicates that the inhibition is mediated by activation of presynaptic mGluRs. Since the effects of L-AP4 can be mimicked by L-SOP, and these two agonists are selective for Group III mGluRs, members of this mGluR group are implicated as the mediators of the presynaptic inhibition (Schoepp, *Neurochem. Int.* *24*:439, 1994; Pin and Duvoisin, *Neuropharmacology* *34*:1). In olfactory bulb neurons it has been demonstrated that L-AP4 activation of mGluRs inhibits presynaptic calcium currents (Trombley and Westbrook, *J. Neurosci.* *12*:2043, 1992). It is therefore likely that the mechanism of presynaptic inhibition produced by activation of Group III mGluRs is similar to that for Group II mGluRs, i.e. blockade of N-type calcium channels and inhibition of neurotransmitter release. L-AP4 is also known to act postsynaptically to hyperpolarize ON bipolar

cells in the retina. It has been suggested that this action may be due to activation of a mGluR, which is coupled to the cGMP phosphodiesterase in these cells (Schoepp, *Neurochem. Int.* *24*:439, 1994; Pin and Duvoisin, *Neuropharmacology* *34*:1).

Metabotropic glutamate receptors have been implicated as playing roles in a number of normal processes in the mammalian CNS. Activation of mGluRs has been demonstrated to be a requirement for the induction of hippocampal long-term potentiation and cerebellar long-term depression (Bashir *et al.*, *Nature* *363*:347, 1993; Bortolotto *et al.*, *Nature* *368*:740, 1994; Aiba *et al.*, *Cell* *79*:365, 1994; Aiba *et al.*, *Cell* *79*:377, 1994). A role for mGluR activation in nociception and analgesia has also been demonstrated (Meller et al., Neuroreport 4: 879, 1993). In addition, mGluR activation has been suggested to play a modulatory role in a variety of other normal processes including: synaptic transmission, neuronal development, apoptotic neuronal death, synaptic plasticity, spatial learning, olfactory memory, central control of cardiac activity, waking, motor control, and control of the vestibulo-ocular reflex (for reviews, see Nakanishi, Neuron 13: 1031, 1994; Pin and Duvoisin, *Neuropharmacology* *34*:1; Knopfel *et al.*, *J. Med. Chem.* *38*:1417, 1995).

Metabotropic glutamate receptors have also been suggested to play roles in a variety of pathophysiological processes and disease states affecting the CNS. These include stroke, head trauma, anoxic and ischemic injuries, hypoglycemia, epilepsy, and neurodegenerative diseases such as Alzheimer's disease (Schoepp and Conn, *Trends Pharmacol. Sci.* *14*:13, 1993; Cunningham et al., Life Sci. 54:135, 1994; Hollman and Heinemann, *Ann. Rev. Neurosci.* *17*:31, 1994; Pin and Duvoisin, *Neuropharmacology* *34*:1, 1994; Knopfel *et al.*, *J. Med. Chem.* *38*:1417, 1995). Much of the pathology in these conditions is thought to be due to excessive glutamate-induced excitation of CNS neurons.

Since Group I mGluRs appear to increase glutamate-mediated neuronal excitation via postsynaptic mechanisms and enhanced presynaptic glutamate release, their activation probably contributes to the pathology. Therefore, selective antagonists of these receptors could be therapeutically beneficial, specifically as neuroprotective agents or anticonvulsants. In contrast, since activation of Group II and Group III mGluRs inhibits presynaptic glutamate release and the subsequent excitatory neurotransmission, selective agonists for these receptors might exhibit similar therapeutic utilities. Thus, the various mGluR subtypes may represent novel targets for CNS drug development.

Preliminary studies assessing therapeutic potentials with the available mGluR agonists and antagonists have yielded seemingly contradictory results. For example, it has been reported that application of ACPD onto hippocampal neurons leads to seizures and neuronal damage (Sacaan and Schoepp, Neurosci. Lett. 139:77, 1992; Lipparti et al., Life Sci. 52:85, 1993). But, other studies indicate that ACPD can inhibit epileptiform activity (Taschenberger *et al.*, *Neuroreport* 3:629, 1992; Sheardown, *Neuroreport* 3:916, 1992), and can also exhibit neuroprotective properties (Koh *et al.*, *Proc. Natl. Acad. Sci. USA* 88:9431, 1991; Chiamulera et al., Eur. J. Pharmacol. 216:335, 1992; Siliprandi *et al.*, *Eur. J. Pharmacol.* 219:173, 1992; Pizzi *et al.*, *J. Neurochem.* 61:683, 1993). It is likely that these opposing results are due to ACPD's lack of selectivity, and activation of different mGluR subtypes. A reasonable explanation for the results is that Group I mGluRs were activated in the former studies to enhance excitatory neurotransmission, while the latter effects were mediated by activation of Group II and/or Group III mGluRs to inhibit presynaptic glutamate release, and diminish excitatory neurotransmission. The observations that (S)-4C3HPG, a Group I mGluR antagonist and Group II mGluR agonist,

protects against audiogenic seizures in DBA/2 mice (Thomsen *et al.*, *J. Neurochem.* *62*:2492, 1994); while the Group II mGluR selective agonists DCG-IV and L-CCG-I protect neurons from NMDA- and KA-induced toxicity (Bruno *et al.*, *Eur. J. Pharmacol.* *256*:109, 1994; Pizzi *et al.*, *J. Neurochem.* *61*:683, 1993) are also consistent with this interpretation.

It is evident that the currently available mGluR agonists and antagonists are of limited use, both as research tools and potential therapeutic agents, as a result of their lack of potency and selectivity. In addition, since these compounds are for the most part amino acids or amino acid derivatives, they have limited bioavailibities which hampers *in vivo* studies assessing mGluR physiology, pharmacology and therapeutic potential. The identification of agonists, antagonists, allosteric modulators and the like with a high degree of potency and selectivity for individual mGluR subtypes is therefore the most important requirement to increase the understanding of various mGluRs' roles in physiological and pathophysiological processes in the mammalian CNS (Pin and Duvoisin, *Neuropharmacology* *34*:1, 1994; Knopfel *et al.*, *J. Med. Chem.* *38*:1417, 1995). Such molecules or lead compounds would serve as important templates for extensive chemical modification studies to further improve potency, mGluR subtype selectivity, and important therapeutic characteristics such as bioavailibility.

It is now evident that the natural agonist (glutamate) binding site in mGluRs is within the unusually large ECD (O'Hara *et al.*, *Neuron* *11*:41, 1993; Takahashi *et al.*, *J. Biol. Chem.* *268*:19341, 1993; Shigemoto *et al.*, *Neuron* *12*:1245, 1994). All mGluR agonists and antagonists currently known in the art are thought to bind at this glutamate binding site. Only one other G-protein coupled receptor, the calcium receptor, has an ECD with structural homology to the ECD of mGluRs (Brownet *al.*, *Nature* *366*:575, 1993; Knopfel *et al.*, *J. Med. Chem.* *38*:1417,

1995). The natural agonist for this receptor is the inorganic ion, calcium. Although the organic molecule glutamate is clearly structurally distinct from calcium, the agonist binding site(s) for calcium are similarly within the large ECD of the calcium receptor (Pollak *et al.*, *Cell 75*:1297, 1993; Yah-Huei *et al.*, *Am. J. Hum. Genet. 56*:1075, 1995; Brown *et al.*, *Eur. J. Endocrinology 132*:523, 1995; Hammerland *et al.*, *J. Bone and Mineral Res. 10*:S156, 1995) In fact, it has now been demonstrated that the ECD of these two distinct but related receptors can be exchanged creating functional chimeric receptors in which agonist activation is primarily determined by which ECD is present (Hammerland *et al.*, *J. Bone and Mineral Res. 10*:S156, 1995). Thus mGluRs and calcium receptors are not only structurally similar but, are functionally similar as well. Compounds which act to modulate calcium receptor activity have now been described which are positive allosteric modulators and are therefore thought to act at a site distinct from the natural agonist (calcium) binding site(s) (Steffey *et al.*, *J. Bone and Mineral Res. 8*:S175, 1993).

None of the references mentioned herein are admitted to be prior art to the claims.

Summary of the Invention

The present invention concerns compounds and methods for modulating metabotropic glutamate receptor activity and binding to metabotropic glutamate receptors. The compounds are targeted to a binding site on the metabotropic glutamate receptor which appears to be distinct from the glutamate binding site. The preferred use of the compounds and methods of the present invention is to modulate metabotropic glutamate receptor activity and thereby aid in the treatment of neurological diseases or disorders. The compounds and methods can also be used to produce other effects such as an analgesic effect,

cognition-enhancement effect, and a muscle-relaxant effect.

Examples are provided of methods of identifying compounds which modulate metabotropic glutamate receptor activity by binding to a novel site on metabotropic glutamate receptors. This site is distinct from the binding site of the natural agonist, glutamate. Such compounds may be structurally similar to compounds which modulate calcium receptor activity by binding to a novel site on the calcium receptor which is distinct from the binding site of the natural agonist, calcium. The binding of such compounds to both metabotropic glutamate and calcium receptors is expected based on the significant amino acid sequence homology between these receptors. However, many such compounds are expected to display differential affinity (pharmacological specificity) for the various metabotropic glutamate and calcium receptors due to amino acid sequence differences between these receptors.

Those in the art will appreciate that a commonality exists between the natural agonist binding sites of numerous related receptors, that is, receptors which exhibit significant amino acid sequence homology. This is well documented for the biogenic amine receptors which have related amino acid sequences. For example, based on laboratory data and predictive methods, molecular models have been developed which reveal common receptor features involved in the binding interactions of biogenic amines (dopamine, adrenaline, serotonin, acetylcholine, etc.) within the 7TMDs of their respective receptors (Hilbert et al., Mol. Pharmacol., 40:8, 1991; Trumpp-Kallmeyer et al., J. Med. Chem., 35:3448, 1992; Baldwin, EMBO J., 12:1693, 1993). A common molecular mechanism by which agonist binding induces signal transduction has also been proposed based on conservation of amino acid sequences within the 7TMD (Oliveira, et al., Trends in Pharmacological Sci., 15:170, 1994).

The commonality of features within the agonist binding pocket of biogenic amine receptors reflects the amino acid sequence homology between receptors of this class and is the basis of receptor "cross-talk". By "cross-talk" is meant the ability of a single compound to interact with more than one receptor. Numerous examples of such "cross-talk" are known in the art. For example, compounds are known in the art which cross-talk between cholinergic, histaminergic and adrenergic receptors (Richelson, et al., J. Clin. Psychiatry, 45:331, 1984), and others which cross-talk between seratonergic and dopaminergic receptors (Foreman et al., J. Pharmacol. Exp. Ther. 260:51, 1992) and still others which cross-talk between cholinergic, adrenergic, dopaminergic and seratoninergic receptors (Roth et al., J. Pharmacol. Exp. Ther., 260:1361, 1992). These examples are but a few of many known to those in the art and are not meant to be limiting.

By way of further example, alpha-2 and beta-2 adrenergic receptors share about 25% amino acid sequence identity and both bind to adrenalin-related compounds, including various agonists and antagonists. The binding of related compounds by these two receptors reflects a similarity in the amino acids which define the natural agonist binding pockets of the receptors.

Some compounds like the agonist, epinephrine, display almost equal affinity for beta-2 and alpha-2 adrenergic receptors. However, other related compounds bind one receptor with an affinity more than 10,000 times greater than they bind the other receptor and thus exhibit pharmacological specificity for one of the two receptors. It has been demonstrated that a single amino acid difference between alpha-2 and beta-2 receptors within the 7TMD plays a major role in determining the differential binding of such related compounds to these two adrenergic receptors (Suryanarayana, et al., J. Biol. Chem., 266:15488, 1991). Differential affinities (pharmacological specificities) displayed by structurally

related compounds therefore reflect specific amino acid sequence differences within the binding pockets of the receptors which amino acids interact differentially with the different substituents on the various structurally-related compounds.

Thus, the structural features of compound binding pockets may be homologous enough to allow similar binding of, for example, native agonists to related receptors. However, derivatives and synthetic analogs of such compounds may exhibit pharmacological specificity (differential affinity) amongst the related receptors due to specific amino acid differences in the sequences comprising the compound binding pockets of the related receptors. Once lead compounds are identified which bind to such compound binding pockets, those skilled in the art can design derivatives and synthetic analogs of such compounds which are targeted to these binding pockets and determine which of the analogs or derivatives are more potent at individual receptors.

Metabotropic glutamate receptors do not exhibit significant amino acid sequence homology with biogenic amine receptors, or with any other class of G-protein coupled receptors except for the calcium receptor. Consistent with this lack of amino acid sequence homology, no natural agonists for any other G-protein coupled receptor types are known to interact with metabotropic glutamate receptors and glutamate does not appear to interact with other G-protein coupled receptors. All metabotropic glutamate receptor agonists and antagonists currently known in the art are amino acids or derivatives thereof. These analogs exhibit differential affinity for the various metabotropic receptor subclasses as described herein in the background section. Thus a commonality of the features comprising the natural agonist binding sites of the various mGluRs exists which reflects the amino acid sequence homology (about 40-70% identity) within the extracellular domain (ECD) of these receptors. Yet the

amino acid sequence differences between metabotropic glutamate receptor subtypes provides for pharmacological differentiation by structurally-related compounds.

The calcium receptor exhibits structural relatedness to metabotropic glutamate receptors which is reflected in amino acid sequence homology of about 25% identity for both ECD and 7TMD regions. The failure of the native agonists glutamate and calcium to cross-talk between these two receptor types is similar to the pharmacological specificity exhibited by serotonin for seratonergic receptors and dopamine for dopaminergic receptors. Yet the sequence conservation between calcium and metabotropic glutamate receptors suggests that some non-native compounds may cross-talk just as some synthetic compounds cross-talk between seratonergic and dompaminergic receptors. In fact, polyamines which activate the calcium receptor (Nemeth and Scarpa, "Spermine Evokes the Rapid Mobilization of Cellular Ca^{2+} in Parathyroid Cells," in Calcium Binding Proteins in Health and Disease, 33 (Academic Press, 1987) are also positive modulators of metabotropic glutamate receptor activity (Alexander et al. J. Neurochem., 59:610, 1992, Manev et al., NeuroReport, 4:830, 1993).

Thus, it is an aspect of the present invention that a compound which binds to the calcium receptor at a site distinct from the calcium binding site(s) and modulates calcium receptor activity defines a structural class of compounds some members of which may bind to metabotropic glutamate receptors at a site distinct from the glutamate binding site and modulate metabotropic glutamate receptor activity. Different structural classes of compounds can be identified which bind calcium receptors and metabotropic glutamate receptors at the same or different novel sites. The availability of cloned calcium receptors allows those skilled in the art to identify compounds of the invention which modulate metabotropic glutamate receptor activity by first identifying compounds and/or

structural classes of compounds which modulate calcium receptor activity.

Compounds so identified can serve as lead compounds to identify different structural classes of molecules which bind the same site on a metabotropic glutamate receptor by using, for example, competitive binding assays known to those in the art but employing labeled compounds of the present invention. Individual compounds within each structural class of compounds are expected to exhibit differential affinities for the calcium receptor or for metabotropic glutamate receptors, subclasses of metabotropic glutamate receptors and even for individual metabotropic glutamate receptors. Preferred compounds are those that bind metabotropic glutamate receptors with greater affinity than calcium receptors. Even more preferred molecules bind to a single subclass of metabotropic glutamate receptor with greater affinity than other subclasses of metabotropic glutamate receptors. Most preferred molecules bind an individual metabotropic glutamate receptor with greatest affinity relative to other mGluRs.

Compounds so identified can also serve as lead compounds to identify different structural classes of molecules by using functional assays known to those skilled in the art and as described in the present invention. Individual compounds within each structural class of compounds are expected to exhibit different potencies at individual calcium receptors and metabotropic glutamate receptors, subclasses of metabotropic glutamate receptors and even at individual metabotropic glutamate receptors. Preferred compounds are those that are more potent at metabotropic glutamate receptors than calcium receptors. Even more preferred molecules are more potent at a single subclass of metabotropic glutamate receptors than at other subclasses of metabotropic glutamate receptors. Most preferred molecules are most potent at an individual metabotropic glutamate receptor with greatest

affinity relative to other mGluRs. Other preferred molecules exhibit pharmacological specificity for metabotropic glutamate receptors in general. More preferred molecules exhibit pharmacological specificity for a subclass of metabotropic glutamate receptors and most preferred molecules exhibit pharmacological specificity for individual metabotropic glutamate receptors as described in the examples provided.

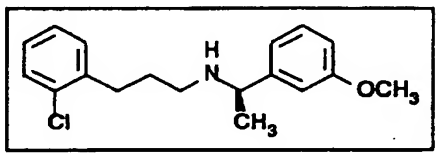
Compounds so identified can also serve as lead compounds for chemical modification by methods well known to those in the art to obtain preferred compounds. Individual compounds within each structural class of compounds are expected to exhibit pharmacological specificity for the calcium receptor or for metabotropic glutamate receptors, metabotropic glutamate receptor subtypes and even for individual metabotropic glutamate receptors. Preferred compounds are those that are more potent at metabotropic glutamate receptors than at calcium receptors. Even more preferred molecules are more potent at a single subclass of metabotropic glutamate receptors than at other subclasses of metabotropic glutamate receptors. Most preferred molecules are most potent at an individual metabotropic glutamate receptor.

A novel class of compounds has been described, the members of which modulate calcium receptor activity (Steffy et al., J. Bone and Mineral Res., 8:5175, 1993; Nemeth et al., PCT Patent Application, International Pub. No. WO93/04373). Generally such compounds are positive allosteric modulators which act at a site on the calcium receptor distinct from the calcium binding site(s). Those in the art will appreciate that structural modifications of such compounds may alter their interaction with the receptor such that they modulate receptor activity differently. For example, such a modified compound binding at the allosteric modulatory site of the calcium receptor, may interact with the receptor such that it inhibits activation of the receptor, or may activate the

receptor in the absence of the natural ligand. Individual compounds of this structural class will thus display different useful properties of receptor modulation such as agonism, antagonism, allosteric modulation and the like by binding to the same site on the calcium receptor or to a homologous site on a metabotropic glutamate receptor. Thus, an aspect of the present invention identifies a class of compounds able to modulate metabotropic glutamate receptor activity by interacting with a binding site which is distinct from the glutamate binding site. The availability of cloned calcium receptors, metabotropic glutamate receptors and chimeric receptors allows those skilled in the art to readily determine which compounds of the class modulate an activity of individual metabotropic glutamate receptors and the type of modulation achieved as described in the examples provided.

The class of compounds of the present invention which are generally allosteric modulators of the calcium receptor do not resemble calcium or glutamate and do not act within the natural agonist binding site(s) of either receptor. Compounds of the class are generally relatively hydrophobic compounds with at least one positive charge; features thought to be important for compound interactions within the 7TMDs of G-protein coupled receptors. Thus, it is a further aspect of the present invention that compounds of the class bind the calcium receptor at a site distinct from the natural agonist binding site(s) and which is likely to be within the 7TMD. Thus, compounds of the class also bind to metabotropic glutamate receptors at a novel site, the mGluR:CaR site, which is distinct from the glutamate binding site and which is homologous to the site on the calcium receptor at which compounds of the class bind and is therefore probably within the 7TMD of the metabotropic glutamate receptor. Such compounds may act as agonists, antagonists, allosteric modulators and the like.

Examples are also provided describing the ability of a compound of the novel class which modulates calcium receptor activity, Compound A, to modulate metabotropic glutamate receptor activity and the use of Compound A as an example of a lead compound to obtain other compounds able to modulate metabotropic glutamate receptor activity. Compound A has the chemical structure:



Compound A acts at a site on the calcium receptor (Compound A binding site) which is distinct from the calcium binding site(s). The structural differences between compounds currently known in the art which modulate metabotropic glutamate receptor activity and Compound A indicate that Compound A binds to a site, the mGluR:CaR site which is distinct from the glutamate binding site. Those in the art will appreciate that other compounds of the class may bind the calcium receptor at the Compound A binding site and/or bind to metabotropic glutamate receptors at the mGluR:CaR site.

Thus, the present invention identifies a class of compounds able to modulate metabotropic glutamate receptor activity by interacting with a binding site which is distinct from the glutamate binding site. Some of these compounds are believed to be structurally and functionally similar to compounds able to act at a calcium receptor binding site. This determination facilitates the development of methods and protocols to obtain additional

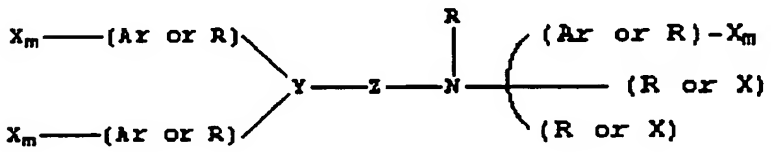
compounds able to modulate metabotropic glutamate receptor activity and/or bind to a metabotropic glutamate receptor. Additional compounds can be identified, for example, by screening for compounds that bind to the metabotropic glutamate receptor at the same binding site as other compounds found to modulate the metabotropic glutamate receptor or bind to the metabotropic glutamate receptor at the novel site (lead compounds) and by determining the biological, pharmacological and/or physiological properties of the compounds.

Modulating metabotropic glutamate receptor activity causes an increase or decrease in a cellular response which occurs upon metabotropic glutamate receptor activation. Cellular responses to metabotropic glutamate receptor activation vary depending upon the type of metabotropic glutamate receptor activated. Generally, metabotropic glutamate receptor activation causes one or more of the following activities: activation of phospholipase C, increase in inositol phosphate, increases in phosphoinositide (PI) hydrolysis, mobilization of intracellular calcium, activation of phospholipase D, activation or inhibition of adenylate cyclase, increases or decreases in the formation of cyclic adenosine monophosphate (cAMP), activation of guanylate cyclase, increases in the formation of cyclic guanosine monophosphate (cGMP), activation of phospholipase A_2, increases in arachidonic acid release, and increases or decreases in the activity of voltage- and ligand-gated ion channels. By "modulating metabotropic glutamate receptor activity" is meant to cause an increase or decrease in an activity of a cellular receptor. The modulation may be caused by a modulator; modulators include agonists, antagonists, allosteric modulators and the like.

Activation of a particular metabotropic glutamate receptor refers to the production of one or more activities associated with the type of receptor activated. Activation of mGluR1 can result in one or more of the

following: increase in PI hydrolysis, increase in cAMP formation and increase in arachidonic acid formation. Compounds can modulate one or more metabotropic glutamate receptor activities by binding to the novel site and acting as an agonist, antagonist, allosteric modulator and the like.

Thus, a first aspect of the present invention describes a method for modulating metabotropic glutamate receptor activity. The method involves contacting a metabotropic glutamate receptor with a sufficient amount of a compound to modulate a metabotropic glutamate receptor activity. The compound has the chemical structure:



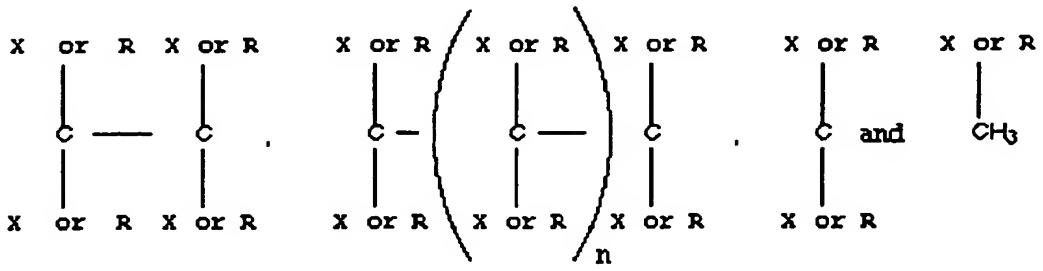
where each X independently is selected from the group consisting of H, CH_3, CH_3O, CH_3CH_2O, methylene dioxy, Br, Cl, F, CF_3, CHF_2, CH_2F, CF_3O, CH_3S, OH, CH_2OH, $CONH_2$, CN, NO_2, CH_3CH_2, propyl, isopropyl, butyl, isobutyl, t-butyl, and acetoxy;

Ar is a hydrophobic entity;

each R independently is selected from the group consisting of hydrogen, methyl, ethyl, propyl, isopropyl, butyl, allyl, isobutyl, t-butyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, indenyl, indanyl, dihydroindolyl, thiodihydroindolyl, 2-, 3-, or 4- piperid(in)yl;

Y is selected from the group consisting of CH, nitrogen and an unsaturated carbon; and

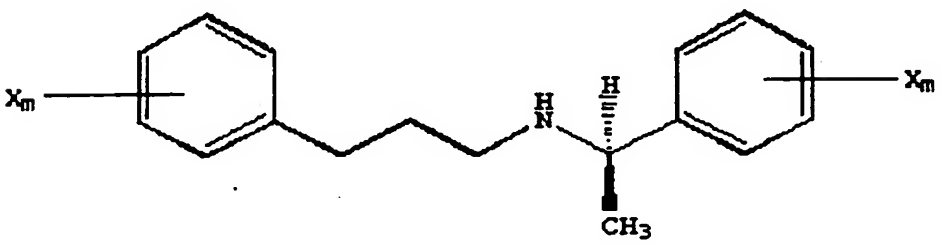
Z is selected from the group consisting of oxygen, nitrogen, sulfur,



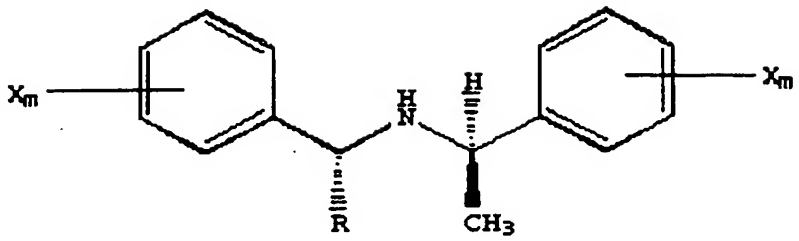
where each n is independently between 1 and 4 inclusive, and each m is independently between 0 and 5 inclusive. Preferably, the compound is a potent agonist or antagonist of a metabotropic glutamate receptor activity.

A hydrophobic entity refers to a non-polar group or moiety such as an aromatic or a cycloaliphatic ring or ring system. Preferably, the hydrophobic entity is selected from the group consisting of phenyl, cyclohexyl, 2-, 3-, or 4-pyridyl, 1- or 2-naphthyl, α- or β-tetrahydronaphthyl, 1- or 2-quinolinyl, 2- or 3-indolyl, benzyl, and phenoxy.

In one embodiment, the compound is a substituted *R*-phenylpropyl-α-phenethylamine derivative, or a substituted *R*-benzyl-α-phenethylamine derivative, having the structure:

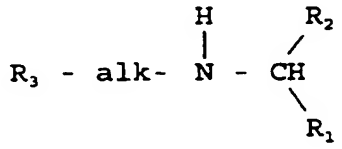


or



with each X preferably being independently selected from the group consisting of Cl, F, CF_3, CH_3, isopropyl, CH_3O, CH_3S, CF_3O, an aliphatic ring and an attached or fused, preferably fused aromatic ring; and R is preferably H, CH_3, ethyl, or isopropyl. Preferably, the aromatic and aliphatic rings have 5 to 7 members. More preferably, the aromatic and aliphatic rings contain only carbon atoms (*i.e.*, the ring in not a heterocyclic ring).

In another embodiment the compound is a substituted *R*-phenylpropyl-α-phenethylamine or substituted *R*-benzyl-α-1-napthylethylamine analogue or derivative, having the structure:



where alk is straight- or branched-chain alkylene of from 0 to 6 carbon atoms;

R_1 is lower alkyl of from 1 to 3 carbon atoms or lower haloalkyl of from 1 to 3 carbon atoms substituted with from 1 to 7 halogen atoms; and

R_2 and R_3 are independently selected carbocyclic aryl or cycloalkyl groups, either monocyclic or bicyclic, having 5- to 7-membered rings optionally substituted with 1 to 5 substituents independently selected from lower alkyl of 1 to 3 carbon atoms, lower haloalkyl of 1 to 3

carbon atoms substituted with 1 to 7 halogen atoms, lower alkoxy of 1 to 3 carbon atoms, halogen, nitro, amino, alkylamino, amido, lower alkylamido of 1 to 3 carbon atoms, cyano, hydroxy, acyl of 2 to 4 carbon atoms, lower hydroxyalkyl of 1 to 3 carbon atoms or lower thioalkyl of 1 to 3 carbon atoms.

Suitable carbocyclic aryl groups are groups having one or two rings, at least one of which has aromatic character and include carbocyclic aryl groups such as phenyl and bicyclic carbocyclic aryl groups such as naphthyl. As is apparent from the above structure, the compound may exist as racemic mixtures containing individual stereoisomers.

Suitable compounds found to modulate metabotropic glutamate receptor activity may be used as lead compounds to screen for additional compounds which modulate metabotropic glutamate receptor activity at sites distinct from the glutamate binding site.

In another aspect, a method is provided for modulating metabotropic glutamate receptor activity comprising the step of contacting said receptor with a compound which modulates the activity of said metabotropic glutamate receptor wherein said compound binds to said receptor and inhibits binding of a compound of the class of compounds above to said receptor.

In preferred aspects, said activities of the metabotropic glutamate receptor are selected from the group consisting of increase in phosphoinositide hydrolysis, activation of phospholipase C, increase in inositol phosphate, increase in intracellular calcium, mobilization of intracellular calcium, activation of adenylate cyclase, inhibition of adenylate cyclase, increase in cAMP formation, decrease in cAMP formation, activation of guanylate cyclase, increase in cGMP formation, activation of phospholipase A_2, increase in the activity of voltage-gated ion channels, decrease in the activity of voltage-gated ion channels, increase in the activity of ligand-gated ion channels, decrease in the

activity of ligand-gated ion channels, activation of phospholipase D, and increase in arachidonic acid formation. In more preferred aspects, said activity comprises a decrease in cAMP formation. In another preferred aspect, said activity comprises an increase in intracellular calcium. In another preferred aspect, said compound is Compound A.

Another aspect of the present invention describes a method for modulating metabotropic glutamate receptor activity by targeting the mGluR:CaR site homologous to the Compound A binding site on the calcium receptor. The method involves the step of contacting a metabotropic glutamate receptor with a compound that binds to the receptor at the mGluR:CaR site and thereby acts as an antagonist, agonist, allosteric modulator or the like. Compounds binding to the mGluR:CaR site can increase an activity of a metabotropic glutamate receptor by binding to the mGluR:CaR site and activating the receptor as for example by acting as agonists, positive allosteric modulators and the like. Compounds binding to the mGluR:CaR site can decrease an activity of a metabotropic glutamate receptor by binding to the mGluR:CaR site and inhibiting the activation of the metabotropic glutamate receptor as for example by acting as antagonists, partial agonists, inverse agonists, negative allosteric modulators and the like.

In another aspect of the invention, a method is provided for modulating metabotropic glutamate receptor activity comprising the step of contacting said receptor with a compound which modulates mGluR activity. In a preferred aspect, the compound also modulates calcium receptor activity. In another preferred aspect, the compound binds to a calcium receptor at a calcium receptor binding site which is distinct from the calcium binding site. In a further preferred aspect, the compound inhibits binding of a molecule to a calcium receptor compound binding site which is distinct from the calcium

binding site. In more preferred aspects, the compound binds to a calcium receptor, preferably to the 7 transmembrane domain of a calcium receptor. In other preferred aspects, the compound binds to a metabotropic glutamate receptor at a binding site that is distinct from the glutamate binding site. In another preferred aspect, said compound binds to the seven transmembrane domain of metabotropic glutamate receptor. In another aspect, the metabotropic glutamate receptor binding site is related to a compound binding site on a calcium receptor. By "related to" is meant that the amino acid sequence of the region or regions of the metabotropic glutamate receptor to which the compound binds is homologous (approximately 20% or more sequence identity) to the amino acid sequence of the region or regions of a calcium receptor to which the compound binds. In preferred aspects, the mGluR:CaR site is related to the Compound A binding site on a calcium receptor.

Another aspect of the present invention describes a method for treating a patient having a neurological disease or disorder. The method involves the step of administering to a patient suffering from the disease or disorder a therapeutically effective amount of a compound which modulates metabotropic glutamate receptor activity by binding at the mGluR:CaR site and acting as an agonist, antagonist, allosteric modulator, or the like. In other aspects, the mGluR:CaR site is related to the Compound A binding site on a calcium receptor.

The neurological disease or disorder to be treated is preferably selected from the group consisting of neurodegenerative diseases, glutamate excitotoxicity, global and focal ischemic and hemorrhagic stroke, head trauma, spinal cord injury, hypoxia-induced nerve cell damage, and epilepsy. In a preferred embodiment the treated neurodegenerative disease or disorder is Alzheimer's disease, Parkinson's disease or Huntington's disease.

The present invention also provides a method for treating a patient having a neurological disease or disorder, comprising the step of administering to said patient a therapeutically effective amount of a compound which modulates metabotropic glutamate receptor activity, wherein said compound binds to a metabotropic glutamate receptor compound binding site that is distinct from the glutamate binding site. In preferred aspects, said compound also modulates calcium receptor activity. In other preferred aspects, the compound binds to a calcium receptor at a calcium receptor binding site which is distinct from the calcium binding site. In a more preferred aspect, the compound inhibits binding of a molecule to a calcium receptor compound binding site which is distinct from the calcium binding site. In further preferred aspects, said compound binds to the 7 transmembrane domain of a calcium receptor. In more preferred aspects, said compound binds to the 7 transmembrane domain of a metabotropic glutamate receptor. In other preferred aspects, said metabotropic glutamate receptor compound binding site is related to a compound binding site on a calcium receptor. In other preferred aspects, said compound binds to said receptor and inhibits the binding of a compound of the invention of the class described herein to said receptor. In more preferred aspects, said neurological disease or disorder is selected from the group consisting of; glutamate excitotoxicity, global and focal ischemic and hemorrhagic stroke, head trauma, spinal cord injury, hypoxia-induced nerve cell damage, and epilepsy. In more preferred aspects, said neurological disease or disorder is selected from the group consisting of: Alzheimer's disease, Parkinson's disease and Huntington's disease. In other preferred aspects, said compound increases mGluR activity. In further preferred aspects, said compound decreases mGluR activity. In further preferred aspects, said compound modulates one or more activities of an mGluR1 receptor.

In one aspect of the invention, a method is provided for producing an analgesic effect in a patient in need of such treatment comprising the step of administering to said patient a therapeutically effective amount of a compound which modulates metabotropic glutamate receptor activity thereby producing said analgesic effect, wherein said compound also binds to said mGluR at a site distinct from the glutamate binding site.

In another aspect, a method is provided for enhancing cognitive abilities in a patient in need of such treatment comprising the step of administering to said patient a therapeutically effective amount of a compound which modulates metabotropic glutamate receptor activity thereby enhancing cognitive abilities, wherein said compound also binds to said mGluR at a site distinct from the glutamate binding site.

In a further aspect, a method is provided for reducing muscle tension in a patient in need of such treatment comprising the step of administering to said patient a therapeutically effective amount of a compound which modulates metabotropic glutamate receptor activity thereby reducing muscle tension, wherein said compound also binds to said mGluR at a site distinct from the glutamate binding site.

Other aspects of the present invention describe methods for producing in a patient in need of such treatment an analgesic effect; enhancing cognitive abilities; and producing a muscle-relaxant effect. These methods involve the step of administering to a patient a therapeutically effective amount of a compound modulating metabotropic glutamate receptor activity. The compound is targeted to the mGluR:CaR site and its binding modulates a metabotropic glutamate receptor activity.

Another aspect of the present invention describes a method for treating a patient having a neurological disease or disorder by administering to a patient suffering from the disease or disorder a therapeutically

effective amount of a compound having a chemical structure as defined above. The compound modulates one or more activities of a metabotropic glutamate receptor.

In preferred aspects, said activities are selected from the group consisting of increase in phosphoinositide hydrolysis, activation of phospholipase C, increase in inositol phosphate, increase in intracellular calcium, mobilization of intracellular calcium, activation of adenylate cyclase, inhibition of adenylate cyclase, increase in cAMP formation, decrease in cAMP formation, activation of guanylate cyclase, increase in cGMP formation, activation of phospholipase A_2, increase in the activity of voltage-gated ion channels, decrease in the activity of voltage-gated ion channels, increase in the activity of ligand-gated ion channels, decrease in the activity of ligand-gated ion channels, activation of phospholipase D, and increase in arachidonic acid formation. In more preferred aspects, said activity comprises a decrease in cAMP formation. In further preferred aspects, said activity comprises an increase in intracellular calcium. In more preferred aspects said compound also binds to a calcium receptor. In other preferred aspects, said compound is Compound A.

Other aspects of the present invention describe methods for producing in a patient an analgesic effect, enhancing cognitive abilities, and producing a muscle relaxant effect, by administering to a patient in need of such treatment a therapeutically effective amount of a compound having a chemical structure as described above. The compound modulates one or more activities of a metabotropic glutamate receptor.

Another aspect of the present invention describes agents (*e.g.*, compounds and pharmaceutical compositions) able to bind to a metabotropic glutamate receptor at a site distinct from the glutamate binding site. Preferably, the agent can modulate metabotropic glutamate receptor activity.

Other aspects of the present invention describe methods for screening for compounds able to bind a metabotropic glutamate receptor at the mGluR:CaR site.

Other embodiments of the present invention provide methods for screening for compounds able to bind a metabotropic glutamate receptor at the mGluR:CaR site and thereby modulate metabotropic glutamate receptor activity as for example by acting as agonists, antagonists, allosteric modulators and the like.

Further aspects of the present invention describe methods for screening for compounds able to bind a metabotropic glutamate receptor and thereby modulate the effects of compounds binding to the mGluR:CaR site on the metabotropic glutamate receptor.

In one aspect of the invention, a method is provided for identifying a compound able to modulate a metabotropic glutamate receptor activity comprising the steps of: a) identifying compounds which modulate one or more activities of a calcium receptor, b) obtaining structurally related compounds to the compounds identified in step a), and c) identifying those compounds from steps a) and b) which modulate one or more activities of a metabotropic glutamate receptor. Structurally related compounds include, for example, the class of compounds illustrated in Figure 1. Also included are other compounds known to those skilled in the art with similar structural relationships to each other as those of the compounds in Figure 1. In addition, by practicing step a), new compounds other than the compounds of Figure 1 will be identified that bind to the same or different calcium receptor compound binding site as the compounds of Figure 1. Once those compounds are identified, those of ordinary skill in the art can identify compounds structurally related to these new compounds. These structurally related compounds may have similar structural relationships to each other as do the compounds of Figure 1 to each other. Those of ordinary skill in the art

understand that compounds that are structurally related include compound analogs and homologs including compounds wherein, for example, one halide is substituted for another, one aromatic group is substituted for another, or one alkyl chain is substituted for another alkyl chain of a shorter or longer length.

In another aspect of the invention, a method is provided for identifying a compound able to modulate a metabotropic glutamate receptor activity comprising the steps of: a) identifying a first compound which binds to a calcium receptor at a compound binding site distinct from the calcium binding site, b) identifying second compounds which inhibit the binding of said first compound to said calcium receptor by binding to said calcium receptor at a compound binding site distinct from the calcium binding site, and c) identifying those first and second compounds of steps a) and b) which modulate one or more activities of a metabotropic glutamate receptor.

Preferred compounds are more potent at a metabotropic glutamate receptor than at a calcium receptor. Preferred compounds are 10-fold more potent, more preferably 100-fold more potent, and most preferably 1000-fold more potent at a metabotropic glutamate receptor than at a calcium receptor. Preferred compounds are not effective at modulating calcium receptor activity at concentrations below 100 μM, more preferably not below 10 μM and most preferably not below 1 μM.

In another aspect of the invention, a method is provided for identifying a compound able to modulate a metabotropic glutamate receptor comprising the steps of: a) identifying compounds which bind to a calcium receptor, b) obtaining structurally related compounds to the compounds identified in step a), and identifying those compounds of steps a) and b) which modulate one or more activities of a metabotropic glutamate receptor. In preferred aspects, the compounds identified in step a) are

those compounds which bind to the 7 transmembrane domain of a calcium receptor.

To "identify" in the sense of identifying compounds which modulate a receptor or identifying compounds which bind to a receptor is meant to first measure the ability of a compound to modulate an activity of a receptor and/or bind to a receptor according to the methods known to those of ordinary skill in the art and to the methods described herein. Compounds which either modulate and/or bind to a receptor according to such methods of measuring are deemed to be "identified."

In another aspect of the invention, a method is provided for screening for a test compound able to modulate a metabotropic glutamate receptor comprising the steps of: a) providing to said receptor a compound which binds to a binding site on a metabotropic glutamate receptor wherein said binding site is distinct from the glutamate binding site, b) comparing the ability of said compound and said test compound to bind to said metabotropic glutamate receptor, and c) measuring the ability of said test compound which is able to bind to said metabotropic glutamate receptor to modulate metabotropic glutamate receptor activity. In preferred aspects, said compound also binds to a calcium receptor. Preferably, said compound binds to the 7 transmembrane domain of a calcium receptor. In other preferred aspects, said test compound inhibits the binding of a compound of claim 1 to said metabotropic glutamate receptor.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of the Drawings

Figure 1 shows the chemical structures of compounds derived from diphenylpropyl-α-phenethylamine illustrating a family of compounds which can be prepared and screened to find the useful compounds of the invention.

Figure 2 depicts a reaction scheme for the preparation of fendiline or fendiline analogues or derivatives depicted in Figure 1.

Description of the Preferred Embodiments

The present invention concerns compounds and methods for modulating metabotropic glutamate receptor activity. The compounds preferably act as an agonist, antagonist, allosteric modulator, or the like of one or more metabotropic glutamate receptor activities. By modulating metabotropic glutamate receptor activities different effects can be produced, such as anticonvulsant effects, neuroprotectant effects, analgesic effects and cognition-enhancement effects.

Compounds of the present invention modulating metabotropic glutamate receptor activity are defined functionally by their ability to exert an effect, or inhibit the exertion of an effect, normally by a receptor agonist such as glutamate.

I. Definitions

The following is a list of some of the definitions used in the present disclosure. These definitions are to be understood in light of the entire disclosure provided herein.

By "adjunct in general anesthesia" is meant a compound used in conjunction with an anesthetic agent which decreases the ability to perceive pain associated with the loss of consciousness produced by the anesthetic agent.

By "allodynia" is meant pain due to a stimulus that does not normally provoke pain.

By "analgesic" is meant a compound capable of relieving pain by altering perception of nociceptive stimuli without producing anesthesia resulting in the loss of consciousness.

By "analgesic activity" is meant the ability to reduce pain in response to a stimulus that would normally be painful.

By "anticonvulsant activity" is meant efficacy in reducing convulsions such as those produced by simple partial seizures, complex partial seizures, status epilepticus, and trauma-induced seizures such as occur following head injury, including head surgery.

By "binds to or modulates" is meant that the agent may both bind to and modulate the activity of a receptor or the agent may either bind to or modulate the activity of a receptor.

By "calcium binding site" is meant the site

By "calcium binding site" is meant the site or sites on a calcium receptor within the ECD where calcium binds, thereby activating the calcium receptor. If a compound competes with calcium for binding to a calcium receptor and calcium competes with said compound for binding to the calcium receptor and the competition is not due to steric hindrance or allosteric changes at the calcium binding site, then said compound is considered to be a compound that binds at the calcium binding site. If the compound does not bind at the calcium binding site, and said compound binds to a binding site on the calcium receptor, then said compound binds at a site distinct from the calcium binding site. If there is more than one calcium binding site, then the compound binds at a site distinct from all calcium binding sites.

By "causalgia" is meant a painful disorder associated with injury of peripheral nerves.

By "central pain" is meant pain associated with a lesion of the central nervous system.

By "chimeric receptor" is meant a receptor comprising one or more portions of an mGluR and one or more portions of a different mGluR or a calcium receptor. By "cognition-enhancement activity" is meant the ability to improve the acquisition of memory or the performance of a

learned task. Also by "cognition-enhancement activity" is meant the ability to improve normal rational thought processes and reasoning.

By "cognition enhancer" is meant a compound capable of improving learning and memory.

By "Compound A binding site" is meant the site on a calcium receptor to which Compound A binds.

By "efficacy" is meant that a statistically significant level of the desired activity is detectable with a chosen compound; by "significant" is meant a statistical significance at the $p < 0.05$ level.

By "glutamate binding site" is meant the site on an mGluR within the ECD where glutamate binds, thereby activating the mGluR. If a compound competes with glutamate for binding to an mGluR, and glutamate competes with said compound for binding to the mGluR and the competition is not due to steric hindrance or allosteric changes at the glutamate binding site, then said compound is considered to be a compound that binds at the glutamate binding site. If the compound does not bind at the glutamate binding site, and said compound binds to a binding site on the mGluR, then said compound binds at a site distinct from the glutamate binding site.

By "hyperalgesia" is meant an increased response to a stimulus that is normally painful.

By "minimal" is meant that any side effect of the drug is tolerated by an average individual, and thus that the drug can be used for therapy of the target disease or disorders. Such side effects are well known in the art. Preferably, minimal side effects are those which would be regarded by the FDA as tolerable for drug approval for a target disease or disorder.

By "modulate" is meant to cause an increase or decrease in an activity of a cellular receptor.

By "modulator" is meant a compound which modulates a receptor, including agonists, antagonists, allosteric

modulators, and the like. Preferably, the modulator binds to the receptor.

By "more potent" in the context of a compound being more potent at one receptor than at another receptor is meant that the compound modulates one or more activities of one type of receptor with a half-maximal modulatory effect which is at a lower compound concentration relative to the concentration at which the compound exhibits a half-maximal modulatory effect on one or more activities of the other type of receptor. Preferably the difference in compound concentration is 10-fold, more preferably 50-fold, and most preferably 100-fold.

By "muscle relaxant" is meant a compound that reduces muscular tension.

By "neuralgia" is meant pain in the distribution of a nerve or nerves.

By "neurodegenerative disease" is meant a neurological disease affecting cells of the central nervous system resulting in the progressive decrease in the ability of cells of the nervous system to function properly. Examples of neurodegenerative diseases include Alzheimer's disease, Huntington's disease, and Parkinson's disease.

By "neurological disorder or disease" is meant a disorder or disease of the nervous system. Examples of neurological disorders and diseases include global and focal ischemic and hemorrhagic stroke, head trauma, spinal cord injury, hypoxia-induced nerve cell damage as in cardiac arrest or neonatal distress, and epilepsy.

By "neuroprotectant activity" is meant efficacy in treatment of the neurological disorders or diseases. By "potent" is meant that the compound has an EC_{50} value (concentration which produces a half-maximal activation), or IC_{50} (concentration which produces half-maximal inhibition), or K^d (concentration which produces half-maximal binding) at a metabotropic glutamate receptor, with regard to one or more receptor activities, of less

than 10 μM, more preferably less than 100 nM, and even more preferably less than 1 nM.

By "selective" is meant that the compound modulates and/or binds to a metabotropic glutamate receptor at a lower concentration than that at which the compound modulates and/or binds to an ionotropic glutamate receptor. Preferably, the concentration difference is a 10-fold, more preferably 50-fold, and even more preferably 100-fold.

By "therapeutically effective amount" is meant an amount of a compound which produces the desired therapeutic effect in a patient. For example, in reference to a disease or disorder, it is the amount which reduces to some extent one or more symptoms of the disease or disorder, and returns to normal, either partially or completely, physiological or biochemical parameters associated or causative of the disease or disorder. When used to therapeutically treat a patient it is an amount expected to be between 0.1 mg/kg to 100 mg/kg, preferably less than 50 mg/kg, more preferably less than 10 mg/kg, more preferably less than 1 mg/kg. Preferably, the amount provides an effective concentration at a metabotropic glutamate receptor of about 1 nM to 1 μM of the compound. The amount of compound depends on its EC_{50} (IC_{50} in the case of an antagonist) and on the age, size, and disease associated with the patient.

II. The mGluR:CaR Site

The distinctiveness of the mGluR:CaR binding site and the glutamate binding site is evident based on the structures of the various glutamate receptor agonists and antagonists known in the art. The Compound A binding site on the calcium receptor is apparently a binding site distinct from the calcium binding site(s) and can be targeted to modulate calcium receptor activity (Nemeth et al., "Calcium Receptor Active Molecule" PCT Application, International Publication Number WO 93/0437-3). Because

of the amino acid sequence homology between metabotropic glutamate receptors and the calcium receptor, metabotropic glutamate receptors have many of the same features of the calcium receptor which define the binding interactions between the calcium receptor and Compounds of the class like Compound A. These features of metabotropic glutamate receptors thus define a novel binding site on metabotropic glutamate receptors, the mGluR:CaR site, which is homologous to the Compound A binding site on the calcium receptor.

The mGluR:CaR site is apparently a binding site distinct from the glutamate binding site and can be targeted to modulate metabotropic glutamate receptor activity. The metabotropic glutamate receptor agonist and antagonist compounds described in the scientific literature are related to the endogenous agonist, glutamate (for reviews see: Cockcroft *et al.*, *Neurochem. Int.* *23*:583-594, 1993; Schoepp and Conn, *TIPS* *14*:13-20, 1993; Hollmann and Heinemann, *Annu. Rev. Neurosci.* *17*:31-108, 1994; Pin and Duvoisin, Neuropharmacology, 34:1, 1995; and Knopfel et al., J. Med. Chem., 38:1417, 1995). Such agonist and antagonist compounds have an acidic moiety, usually a carboxylic acid, but sometimes a phosphatidic acid. Presumably then, such compounds bind metabotropic glutamate receptors at the same site as the amino acid, glutamate. This has been confirmed, for example, for methylcarboxyphenylglycine, which was shown to be a competitive antagonist of glutamate (Eaton *et al.*, *Eur. J. Pharm. - Mol. Pharm. Sect.* *244*:195-197, 1993). It can be assumed that compounds active at metabotropic glutamate receptors lacking negative charges, and not resembling the amino acid glutamate, do not act at the glutamate binding site.

Those in the art will also appreciate that the Compound A binding site and the mGluR:CaR site are defined by homologous, but not identical, amino acid sequences. Thus, individual compounds of the class may bind calcium

receptors and metabotropic glutamate receptors with different affinities and may exhibit differential potency for modulation of receptor activity. It is possible therefore that binding to one or more receptors may occur with such low affinity that it cannot be detected by practical means. It is also possible that modulation of activity of one or more receptors may be of such low potency that it cannot be detected by practical means. Similarly, mGluR:CaR sites on different metabotropic glutamate receptors may be defined by homologous, but not identical, amino acid sequences. Thus, compounds of the invention which exhibit differences in potency and/or binding affinity at different metabotropic receptors are useful to target specific metabotropic glutamate receptors or groups of receptors comprising metabotropic glutamate receptor subclasses.

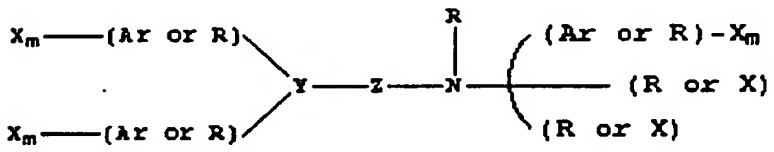
Thus, compounds of the present invention define a binding site on metabotropic glutamate receptors, the mGluR:CaR site, distinct from that of glutamate. Compounds which modulate metabotropic glutamate receptor activity by binding to the mGluR:CaR site will resemble compounds of the class including Compound A.

A. Compounds Which Modulate Metabotropic Glutamate Receptor Activity

Compounds which modulate metabotropic glutamate receptor activity have several uses including diagnostic uses and therapeutic use. Potential metabotropic glutamate receptor-modulating compounds are illustrated in Figure 1. The syntheses of many of the compounds is described by Nemeth *et al.*, entitled "Calcium Receptor Active Molecules" International Publication Number WO 93/04373, and in copending applications U.S.S.N. 08/353,784, filed December 8, 1994, and U.S.S.N. 08/484,159, filed June 7, 1995, all hereby incorporated by reference herein. Those compounds binding to a metabotropic glutamate receptor and those compounds

efficacious in modulating metabotropic receptor glutamate activity can be identified using the procedures described herein (for example, see section IV, *infra*). Those compounds which can selectively bind to the metabotropic glutamate receptor can be used diagnostically to determine the presence of the metabotropic glutamate receptor versus other glutamate receptors.

The compounds illustrated in Figure 1 are expected to be able to bind to a metabotropic glutamate receptor and, preferably, be efficacious in modulating metabotropic glutamate receptor activity. These compounds have the following chemical structure:

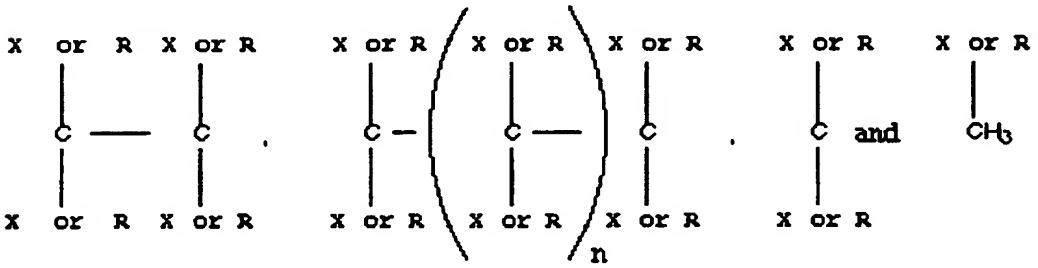


where each X independently is selected from the group consisting of H, CH_3, CH_3O, CH_3CH_2O, methylene dioxy, Br, Cl, F, CF_3, CHF_2, CH_2F, CF_3O, CH_3S, OH, CH_2OH, $CONH_2$, CN, NO_2, CH_3CH_2, propyl, isopropyl, butyl, isobutyl, *t*-butyl, and acetoxy;

Ar is a hydrophobic entity;

each R independently is selected from the group consisting of hydrogen, methyl, ethyl, propyl, isopropyl, butyl, allyl, isobutyl, *t*-butyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, indenyl, indanyl, dihydroindolyl, thiodihydroindolyl, 2-, 3-, or 4- piperid(in)yl;

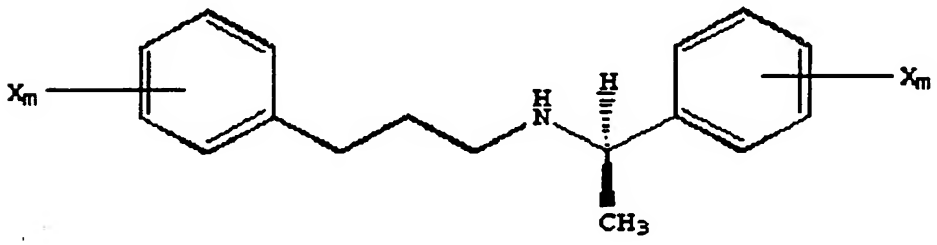
Y is selected from the group consisting of CH, nitrogen and an unsaturated carbon; and Z is selected from the group consisting of oxygen, nitrogen, sulfur,



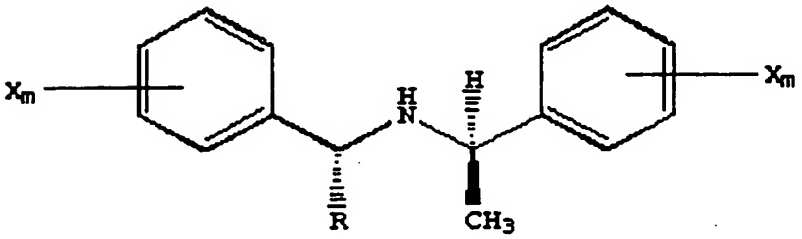
where each n is independently between 1 and 4 inclusive, and each m is independently between 0 and 5 inclusive.

A hydrophobic entity refers to a non-polar group or moiety such as an aromatic or a cycloaliphatic ring or ring system. Preferably, the hydrophobic entity is selected from the group consisting of phenyl, cyclohexyl, 2-, 3-, or 4-pyridyl, 1- or 2-naphthyl, α- or β-tetrahydronaphthyl, 1-or 2- quinolinyl, 2- or 3-indolyl, benzyl, and phenoxy.

In one embodiment the compound is a substituted *R*-phenylpropyl-α-phenethylamine derivative, or a substituted *R*-benzyl-α-phenethylamine derivative having the structure:



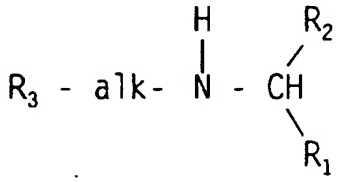
or



with each X preferably being independently selected from the group consisting of Cl, F, CF_3, CH_3, isopropyl, CH_3O, CH_3S, CF_3O an aliphatic ring and an attached or fused, preferably fused aromatic ring; and R is preferably H, CH_3, ethyl, or isopropyl. Preferably, the aromatic and aliphatic rings have 5 to 7 members. More preferably, the aromatic and aliphatic rings contain only carbon atoms (*i.e.*, the ring in not a heterocyclic ring).

Another embodiment of the present invention, provides metabotropic glutamate receptor modulating compounds which are substituted *R*-phenylpropyl-α-phenethylamine and

substituted *R*-benzyl-α-1-napthylethylamine analogues and derivatives, having the structure:



where alk is straight- or branched-chain alkylene of from 0 to 6 carbon atoms;

R_1 is lower alkyl of from 1 to 3 carbon atoms or lower haloalkyl of from 1 to 3 carbon atoms substituted with from 1 to 7 halogen atoms; and

R_2 and R_3 are independently selected carbocyclic aryl or cycloalkyl groups, either monocyclic or bicyclic, having 5- to 7-membered rings optionally substituted with 1 to 5 substituents independently selected from lower alkyl of 1 to 3 carbon atoms, lower haloalkyl of 1 to 3 carbon atoms substituted with 1 to 7 halogen atoms, lower alkoxy of 1 to 3 carbon atoms, halogen, nitro, amino, alkylamino, amido, lower alkylamido of 1 to 3 carbon atoms, cyano, hydroxy, acyl of 2 to 4 carbon atoms, lower hydroxyalkyl of 1 to 3 carbon atoms or lower thioalkyl of 1 to 3 carbon atoms. Suitable carbocyclic aryl groups are groups having one or two rings, at least one of which has aromatic character and include carbocyclic aryl groups such as phenyl and bicyclic carbocyclic aryl groups such as naphthyl. As is apparent from the above structures, preparation of the compounds may exist as racemic mixtures containing individual stereoisomers.

Preferred compounds shown in Figure 1, are Compound A, Compound D, and Compounds 1D, 4L, 8J, 8U, 9R, 11X, 12U, 12V, 12Z, 14U, 16M and 16P. The ability of Compound A to modulate metabotropic glutamate receptor activity is described in the examples below. The other compounds are

also expected to be potent modulators of one or more metabotropic glutamate receptor activity.

B. Synthetic Strategies

Various synthetic strategies can be used to obtain compounds which modulate metabotropic glutamate receptor activity using procedures known in the art. (For example, see Nemeth *et al.*, entitled "Calcium Receptor Active Molecule" International Publication Number WO 93/04373, hereby incorporated by reference herein). An example of a protocol for preparing fendiline (or fendiline analogues shown in Fig. 1), is described below. Chiral resolution may be accomplished using methods such as those described in Example 4.

1. Synthesis of Polyamines

The synthetic methods used to produce polyamines described in this section are modelled after methods used to construct argiopines 636 and 659 and other arylalkyl polyamines derived from spider venoms. Polyamines can be synthesized starting with, for example, diaminoalkanes and simple polyamines such as spermidine or spermine. Strategies for the synthesis and the modification of polyamines involve using a variety of amine-protecting groups (*e.g.*, phthalimido, BOC, CBZ, benzyl, and nitrile) which can be selectively removed to construct functionalized molecules.

Chain extensions, of the starting material, by 2-4 methylenes were typically accomplished by alkylation with the corresponding N-(bromoalkyl)phthalimide. A 1:1.2 mixture of amine to the bromoalkylphthalimide was refluxed in acetonitrile in the presence of 50% KF on Celite. Chain extensions were also accomplished by alkylation of a given amine with acrylonitrile or ethylacrylate. Reaction progress was monitored by thin-layer chromatography (TLC) and intermediates purified on silica gel using combinations of dichloromethane, methanol, and

isopropylamine. Final products were purified by cation exchange (HEMA-SB) and RP-HPLC (Vydac C-18). Purity and structure verification were accomplished by ^{1}H- and ^{13}C-NMR spectroscopy and high-resolution mass spectrometry (EI, CI and/or FAB).

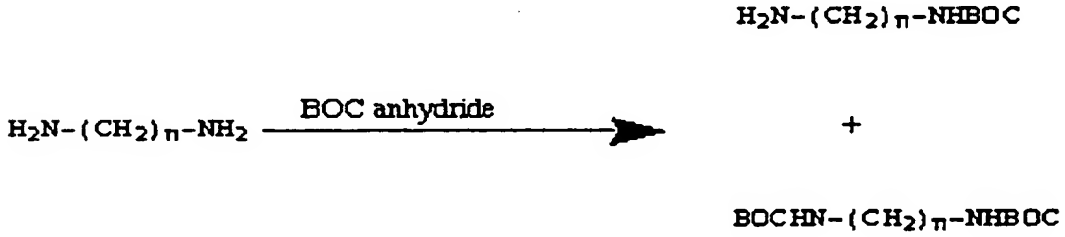
Amine-protecting groups, phthalimido, BOC, CBZ, benzyl, and nitrile, were added and later selectively removed to construct functionalized molecules. BOC protecting groups were added by treating a primary or secondary amine (1° or 2°) with di-tert-butyl dicarbonate in dichloromethane. Benzyl protecting groups were applied in one of two ways: (1) condensation of a 1° amine with benzaldehyde followed by sodium borohydride reduction or (2) alkylation of a 2° amine with benzylbromide in the presence of KF.

Deprotection of the different groups was carried out using different procedures. Deprotection of the phthalimido functionality was accomplished by reduction with hydrazine in refluxing methanol. Deprotection of the BOC functionality was accomplished in anhydrous TFA or concentrated HCl in acetonitrile. Deprotection of benzyl, nitrile, and CBZ protecting functionalities was accomplished by reduction in glacial acetic acid under 55 psi hydrogen in the presence of a catalytic amount of palladium hydroxide on carbon. Nitrile functionalities in the presence of benzyl and CBZ groups were selectively reduced under hydrogen in the presence of sponge Raney nickel.

Amide linkages were typically prepared by reacting an amine (1° or 2°) with an *N*-hydroxysuccinimide or p-nitrophenylester of a given acid. This was accomplished directly, in the case of adding cyclic groups, by treating the amine with dicyclohexylcarbodiimide under dilute conditions.

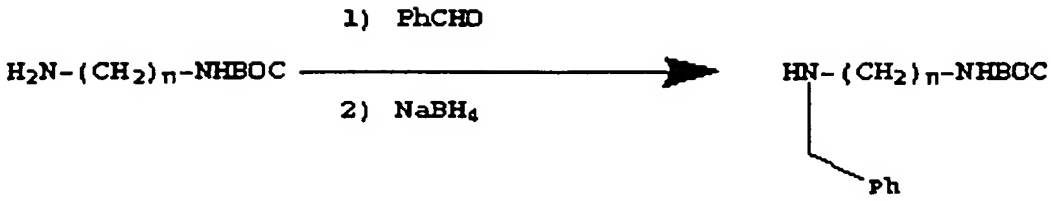
Specifically, branched polyamines are typically prepared from simple diaminoalkanes of the formula NH_2-$(CH_2)_n$-NH_2, or simple polyamines such as spermidine or

spermine. One of the two primary (terminal) amines is protected or "masked" with a protecting group such as BOC (t-butyloxycarbonyl), phthalimido, benzyl, 2-ethylnitrile (the Michael condensation production product of an amine and acrylonitrile), or amide. A typical reaction is the addition of a BOC protecting group by treatment with di-t-butyl-dicarbonate (BOC anhydride):

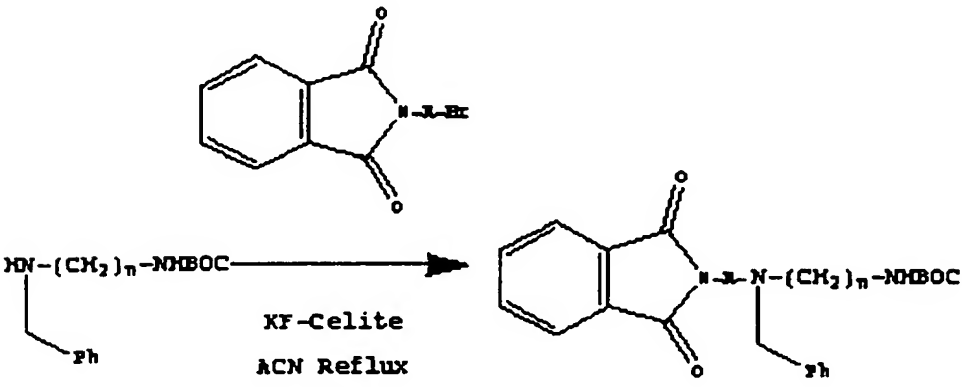


The monoprotected product is separated from the unprotected and diprotected products by simple chromatographic or distillation techniques.

The remaining free amine in the monoprotected product is then selectively alkylated (or acylated) with an alkylating (or acylating) agent. To ensure mono-alkylation, the free amine is partially protected by condensation with benzaldehyde followed by sodium borohydride reduction to form the N-benzyl derivative:

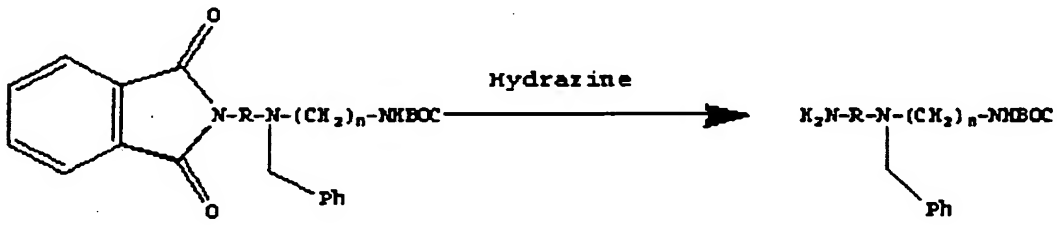


The N-benzyl derivative is then reacted with the alkylating agent. A typical alkylating agent is in an N-(bromoalkyl)phthalimide, which reacts as follows:



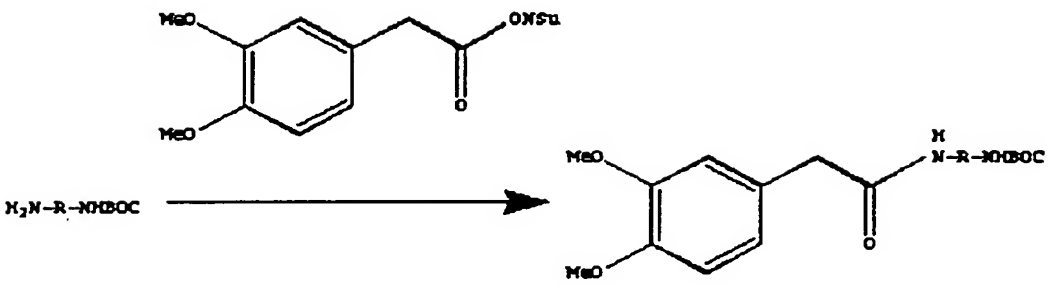
For example, N-(bromobutyl)phthalimide is used to extend or branch the chain with four methylene units. Alternatively, reaction with acrylonitrile followed by reduction of the cyano group will extend the chain by three methylenes and an amino group.

The protecting groups of the resulting chain-extended molecule can then be selectively cleaved to yield a new free amine. For example, trifluoroacetic acid is used to remove a BOC group; catalytic hydrogenation is used to reduce a nitrile functionality and remove a benzyl group; and hydrazine is used to remove phthalimido groups as follows:



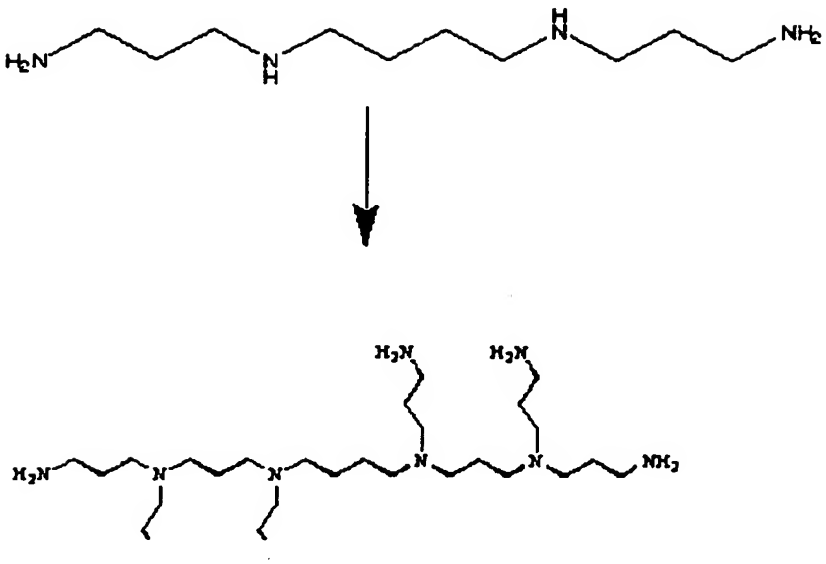
The new free amine may be alkylated (or acylated) further as above to increase the length of the polyamine. This process is repeated until the desired chain length and number of branches is obtained. In the final step, deprotection of the product results in the desired polyamine. However, further modifications may be effected at the protected end prior to deprotection. For example, prior to BOC-deprotection, the polyamine is acylated with the N-hydroxysuccinimide ester of 3,4-dimethoxyphenylacetic acid to yield a diprotected polyamine:

50



This ultimately yields an arylalkyl polyamine. The BOC group can then be selectively removed with trifluoroacetic acid to expose the other amino terminus which can be extended as above.

Certain branched polyamines may be formed by simultaneously alkylating or acylating the free primary and secondary amines in a polyamine formed as above. For example, treatment of spermine with excess acrylonitrile followed by catalytic reduction yields the following:



Cyclic polyamines may be prepared as above with starting materials such as hexacylen (Aldrich Chem.).

2. Polyamino Acid Synthesis

Polyamino acids can be made using standard techniques such as being translated using recombinant nucleic acid techniques or being synthesized using standard solid-phase techniques. Solid-phase synthesis is commenced from the carboxy-terminal end of the peptide using an α-amino protected amino acid. BOC protective groups can be used for all amino groups even through other protective groups

are suitable. For example, BOC-lys-OH can be esterified to chloromethylated polystyrene resin supports. The polystyrene resin support is preferably a copolymer of styrene with about 0.5 to 2% divinylbenzene as a cross-linking agent which causes the polystyrene polymer to be completely insoluble in certain organic solvents. See Stewart *et al.*, Solid-Phase Peptide Synthesis (1969), W.H. Freeman Co., San Francisco; and Merrifield, J. Am. Chem. Soc. (1963) 85:2149-2154. These and other methods of peptide synthesis are also exemplified by U.S. Patent Nos. 3,862,925; 3,842,067; 3,972,859; and 4,105,602.

The polypeptide synthesis may use manual techniques or be automated. For example, synthesis can be carried out using an Applied Biosystems 403A Peptide Synthesizer (Foster City, California) or a Biosearch SAM II automatic peptide synthesizer (Biosearch, Inc., San Rafael, California), following the instructions provided in the instruction manual supplied by the manufacturer.

3. Arylalkyl Polyamines

Arylalkyl polyamines can be obtained from natural sources isolated by known techniques, or synthesized as described in Jasys *et al.*, Tetrahedron Lett. 29:6223-6226, (1988); Nason *et al.*, Tetrahedron Lett. 30:2337-2340, (1989); and Schafer *et al.*, "Polyamine Toxins from Spiders and Wasps," The Alkaloids, vol. 45, p. 1-125, 1994.

4. Arylalkylamines

This section describes general protocol to prepare arylalkylamines such as fendiline or fendiline analogues as shown in Figure 1. In a 10-ml round-bottom flask equipped with a magnetic stir bar and rubber septum, 1.0 mmole 3,3'-diphenylpropylamine (or primary alkylamine such as substituted or unsubstituted phenylpropylamine) in 2 ml ethanol was treated with 1.0 mmole acetophenone (or substituted acetophenone). Two millimoles $MgSO_4$ and 1.0

mmole $NaCNBH_3$ were then added and the solution was stirred under a nitrogen atmosphere at room temperature (about 20°C) for 24 hours. The reaction was poured into 50 ml ether and washed 3 times with 1 *N* NaOH and once with brine. The ether layer was dried with anhydrous K_2CO_3 and reduced *in vacuo*. The product was then purified by column chromatography or HPLC incorporating a silica stationary phase with combinations of CH_2Cl_2-methanol-isopropylamine (typically 3% methanol and 0.1% isopropylamine in methylene chloride).

A preferred procedure for preparing fendiline or fendiline analogues (such as those depicted in Figure 1) uses titanium(IV) isopropoxide and was modified from methods described in J. Org. Chem. 55:2552 (1990). For the synthesis of Compound 2M, titanium tetrachloride (method described in Tetrahedron Lett. 31:5547 (1990)) was used in place of titanium(IV) isopropoxide.

A reaction scheme is depicted in Figure 2. In Figure 2, R, R' and R" depict appropriately substituted hydrocarbon and aromatic moieties groups. Referring to Figure 2 in a 4-ml vial, 1 mmole of amine (1) (typically a primary amine) and 1 mmole ketone or aldehyde (2) (generally an appropriately substituted acetophenone) are mixed, then treated with 1.25 mmoles titanium(IV) isopropoxide (3) and allowed to stand with occasional stirring at room temperature for about 30 minutes. Alternatively, a secondary amine may be used in place of (1). Reactions giving heavy precipitates or solids can be heated to their melting point to allow for mixing several times during the course of the reaction.

The reaction mixture is then treated with 1 ml ethanol containing 1 mmole sodium cyanoborohydride (4) and the resulting mixture is allowed to stand at room temperature with occasional stirring for about 16 hours. After this time the reaction is quenched by the addition of about 500 µl water. The reaction mixture is then diluted to about 4 ml total volume with ethyl ether and then centrifuged.

The upper organic phase is removed and reduced on a rotavapor. The resulting product (6) is partially purified by chromatography through a short silica column (or alternatively by using preparative TLC on silica) using a combination of dichloromethane-methanol-isopropylamine (typically 95:5:0.1), and then purified by HPLC (normal-phase using silica with dichloromethane-methanol-isopropylamine or reversed phase, C-18 with 0.1% TFA with acetonitrile or methanol).

III. Screening For Additional Compounds

The following is a description of procedures which can be used to obtain compounds modulating metabotropic glutamate receptor activity and which are related to Compound A and/or exert an effect at the mGluR:CaR site. The tests are exemplified by the use of Compound A. The use of Compound A in this example is not meant to be limiting and other compounds of the present invention may be used similarly to screen for additional compounds which modulate metabotropic glutamate receptor activity. The use of Compound A as a lead compound is expected to lead to other compounds having similar activity and which in turn can be used as lead compounds. Lead compounds such as Compound A can be used for molecular modeling using standard procedures, and to screen compound libraries.

Radioligand binding techniques (a radiolabeled binding assay) can be used to identify compounds binding at the mGluR:CaR site (see Example 2, *infra*). Data from radioligand binding studies will also confirm that compounds do not inhibit [^{3}H]Compound A binding via an action at the known glutamate binding site on metabotropic glutamate receptors. This screening test allows vast numbers of potentially useful compounds to be screened for their ability to bind to the Compound A binding site. Other rapid assays for detection of binding to the mGluR:CaR site on metabotropic glutamate receptors can be devised using standard detection techniques.

Additional testing utilizing electrophysiological and biochemical methodologies can be carried out to determine the activity of compounds obtained with initial binding assay screening. The additional testing can confirm that a compound binds to the mGluR:CaR site and exerts an effect on metabotropic glutamate receptor activity.

In addition, recombinant DNA technology can be used to facilitate testing (see Example 3, *infra*). Using standard procedures, the gene(s) encoding the amino acids comprising the mGluR:CaR site (*e.g.*, encoding a mGluR1) can be identified and cloned. For example, an affinity column can be prepared using Compound A, and solubilized membranes from cells or tissues containing mGluR1 are passed over the column. The receptor binds to the column and the isolated receptor is subsequently eluted. Partial amino acid sequence information is then obtained allowing for the isolation of the gene encoding the receptor using degenerative probes (for example, see Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1989)).

Alternatively, expression cloning can be used to obtain cloned metabotropic glutamate receptors. cDNA expression libraries are prepared and subfractions of the library are tested for their ability to impart metabotropic glutamate receptor activity on cells which do not normally express such receptors (*e.g.*, CHO cells, mouse L cells, HEK 293 cells, or *Xenopus* oocytes). Metabotropic glutamate receptor activity can be measured, for example by measuring Cl^- currents resulting from PI hydrolysis and Ca^{2+} mobilization. In this way, the library fraction containing the clone encoding the receptor is identified. Sequential subfractionation and assaying activity results in a single clone encoding the metabotropic glutamate receptor.

Once the receptor gene is isolated, standard techniques are used to identify the polypeptide or portion(s) defining the mGluR:CaR site. Further, using

standard procedures, the entire receptor or domains comprising the mGluR:CaR site can be expressed by recombinant technology. The receptor or binding domain(s) can be isolated and used as a biochemical reagent to screen for compounds binding at the Compound A binding site. In this way, large numbers of compounds can be simultaneously screened, *e.g.*, by passage through a column containing the Compound A binding domain, and analysis performed on compounds which bind to the column.

Additional testing utilizes the combination of molecular biological techniques (expression of cloned metabotropic glutamate receptors) and electrophysiological techniques or biochemical techniques to monitor receptor activation. Specifically, compounds can be rapidly screened for potency at cloned and expressed subtypes of metabotropic glutamate receptors.

Amino acid sequences comprising the binding site of a compound of the invention can be identified by additional testing using the combination of molecular biological techniques. For example, using oocytes or cells expressing a chimeric receptor comprising the ECD of the calcium receptor and the 7TMD of mGluR1, and also oocytes or cells expressing a chimeric receptor comprising the ECD of mGluR1 and the 7TMD of the calcium receptor, the potency of the compound at these chimeric receptors can be determined. Compounds which are preferred, that is, more potent at mGluR1 than at the calcium receptor, will exhibit greater potency at the chimeric receptor comprising the mGluR1 domain containing the compound binding site. For example, preferred compounds binding to a site within the 7TMD will be more potent at the chimeric receptor containing the 7TMD of mGluR1. Similarly, if the compound binding site is within the ECD, preferred compounds will be more potent at the chimeric receptor containing the ECD of mGluR1. In this way, the domain containing the preferred compound binding site may be identified. Site directed mutagenesis can be used to

further identify amino acid residues comprising the compound binding site and/or which are important in determining the effects of compounds of the invention.

IV. Assessment of Potency and Identification of Preferred Molecules

Preferred molecules of the invention are more potent at metabotropic glutamate receptors than at the calcium receptor. Potency at the calcium receptor can be assessed in a number of ways using primary isolates of cells or cells expressing the cloned calcium receptor as described (See U.S.S.N. 08/353,784, filed December 9, 1994, hereby incorporated by reference herein). Similarly, potency at metabotropic glutamate receptors can be determined in a number of ways using primary isolates of tissues or cells or cells expressing individual cloned metabotropic glutamate receptors or chimeric receptors. Cell lines expressing metabotropic glutamate receptors have been obtained and methods applicable to their use to identify compounds which modulate activity of metabotropic glutamate receptors and determine potency of same disclosed (European Patent Publication No. 0 568 384 A1; European Patent Publication No. 0 569 240 A1; PCT Publication No. WO 94/29449; PCT Publication No. WO 92/10583; U.S. Pat. No. 5,385,831). Cells expressing chimeric receptors have also been obtained and similar methods are applicable to their use to assess potency of compounds of the invention at the chimeric receptors and thereby at the metabotropic glutamate receptor of interest. (U.S. Patent Application, Fuller et al., "Chimeric Receptors and Methods for Identifying Compounds Active at Metabotropic Glutamate Receptors and the Use of Such Compounds in the Treatment of Neurological Disorders and Diseases," filed July 26, 1995; Hammerland et al., J. Bone and Mineral Res., 10:S156, 1995, both hereby incorporated by reference herein). Thus, recombinant cell-based assays which use biochemical,

spectrophotometric or other physical measurements to detect the modulation of activity of an expressed receptor, especially by measuring changes in affected intracellular messengers, are known to those in the art and can be constructed and treated such that they are suitable for assessing the potencies of compounds of the present invention at individual metabotropic glutamate receptors, calcium receptors and chimeric receptors.

It will also be appreciated by those in the art that each type of method used to assess modulation of metabotropic glutamate receptor activity and potency of compounds of the present invention has advantages and disadvantages which will vary depending on the metabotropic glutamate receptor of interest, the cell lines employed, the nature of the biochemical and physical measurements used to detect modulation of receptor function, the nature of the compound being assessed and various other parameters. An exceptionally useful and practical method is the use of fluorescent indicators of intracellular Ca^{2+} to detect modulation of the activity of receptors coupled to phospholipase-C activation and thereby to the mobilization of intracellular Ca^{2+}.

More preferred compounds may be identified by comparing the potency of a compound at two or more metabotropic glutamate receptors from different subclasses or chimeric receptors comprising appropriate binding domains of same. Most preferred compounds may be identified by similar comparisons of the potency of a compound at each of several metabotropic glutamate receptors. Thus, cells expressing certain metabotropic glutamate receptors or chimeric receptors are preferred for the identification of certain preferred molecules of the invention. For example, chimeric receptors comprising the domain(s) of the calcium receptor required for signal transduction and the domain(s) comprising the compound binding site from individual metabotropic glutamate receptors are expected to exhibit compound binding and

activity modulating properties similar to those of the native metabotropic glutamate receptor. However, these chimeric receptors are preferred over native metabotropic receptors for such assessments because glutamate activation of these chimeras is expected to activate the signal transduction pathways which are associated with activation of the calcium receptor, regardless of which signal transduction pathways are associated with the respective native metabotropic glutamate receptor. Thus, cells expressing such chimeric receptors allow for the assessment of compound potency at any or all metabotropic glutamate receptors and the calcium receptor using the same electrophysiological or biochemical technique.

The following is a description of procedures which can be used to assess potency of compounds of the invention at different metabotropic glutamate receptors and the calcium receptor by using chimeric receptors.

Recombinant Cell Lines Expressing Chimeric Receptors

Nucleic acid expressing a functional chimeric receptor can be used to create transfected cell lines which functionally express a specific chimeric receptor. Such cell lines have a variety of uses such as being used for assessing the potency of compounds of the invention at metabotropic glutamate receptors; and being used to assay binding to a metabotropic glutamate receptor.

A variety of cell lines are capable of coupling exogenously expressed receptors to endogenous functional responses. A number of these cell lines (*e.g.*, NIH-3T3, HeLa, NG115, CHO, HEK 293 and COS7) can be tested to confirm that they lack an endogenous metabotropic glutamate. Those lines lacking a response to external glutamate can be used to establish stably transfected cell lines expressing the cloned chimeric receptors.

Production of these stable transfectants is accomplished by transfection of an appropriate cell line with a eukaryotic expression vector, such as pMSG, in

which the coding sequence for the metabotropic glutamate receptor cDNA has been cloned into the multiple cloning site. These expression vectors contain a promoter region, such as the mouse mammary tumor virus promoter (MMTV), that drive high-level transcription of cDNAS in a variety of mammalian cells. In addition, these vectors contain genes for the selection of cells that stably express the CDNA of interest. The selectable marker in the PMSG vector encodes an enzyme, xanthine-guanine phosphoribosyl transferase (XGPRT), that confers resistance to a metabolic inhibitor that is added to the culture to kill the nontransfected cells. A variety of expression vectors and selection schemes are usually assessed to determine the optimal conditions for the production of chimeric receptor-expressing cell lines and to assess the potencies of compounds of the invention.

The most effective method for transfection of eukaryotic cell lines with plasmid DNA varies with the given cell type. The chimeric receptor expression construct is introduced into cultured cells by the appropriate technique, either Ca^{2+} phosphate precipitation, DEAE-dextran transfection, lipofection or electroporation.

Cells that have stably incorporated or are episomally maintaining the transfected DNA are identified by their resistance to selection media, as described above, and clonal cell lines are produced by expansion of resistant colonies. The expression of the chimeric receptor cDNA by these cell lines may be assessed by solution hybridization and Northern blot analysis. Functional expression of the receptor protein may be determined by measuring the mobilization of intracellular Ca^{2+} in response to externally applied glutamate or calcium.

Measurement of Intracellular Calcium

Measuring intracellular calcium with the calcium indicator, fura-2, provides a very rapid means of assessing the ability of compounds to modulate the

activity of receptors whose activation results in increases or decreases in intracellular calcium levels as for example, receptors coupled to phospholipase-C and thereby to the mobilization of intracellular calcium. The method is applicable to the assessing the potency of compounds of the invention at calcium receptors, at certain metabotropic glutamate receptors, and at certain chimeric receptors, especially those which couple to phospholipase-C. For example, recombinant cells expressing chimeric receptors are loaded with fura-2 and suspended in buffer in a cuvette suitable for use in a fluorometer. A compound is added to the cuvette in a small volume (5-15 μl) and changes in the fluorescence signal are measured. Cumulative increases in the concentration of the compound are made in the cuvette until a maximal practical concentration is reached (i.e., 10 mM compound) or until no further changes in fluorescence are noted. The concentration of compound at which half-maximal fluorescence change occurs is used as the index of potency for the compound at that chimeric receptor. This is expected to be similar or identical to the potency of the compound at the native metabotropic glutamate receptor containing the compound binding site which is present in the chimeric receptor. Similarly, the potency of compounds which inhibit receptor activity can be determined by first stimulating receptor activity by adding a sub-maximal concentration of a natural agonist such as glutamate or calcium and repeating the above compound addition steps to determine the half-maximal inhibitory concentration of compound.

Measurement of cyclic AMP

This section describes measuring cyclic AMP levels. Cells are incubated as above and at the end of the incubation, a 0.15-ml sample is taken and transferred to 0.85 ml of hot (70°C) water and heated at this temperature for 5-10 minutes. The tubes are subsequently frozen and

thawed several times and the cellular debris sedimented by centrifugation. Portions of the supernatant are acetylated and cyclic AMP concentrations determined by radioimmunoassay.

Measurement of Inositol Phosphate Formation

This section describes procedures measuring inositol phosphate formation. Membrane phospholipids are labeled by incubating cells expressing chimeric receptors with 4 μCi/ml ^{3}H-myo-inositol for 20-24 hours. Cells are then washed and resuspended in PCB containing 0.5 mM $CaCl_2$ and 0.1% BSA. Incubations are performed in microfuge tubes in the absence or presence of various concentrations of organic polycation for different times. Reactions are terminated by the addition of 1 ml chloroform-methanol-12 N HCl (200:100:1; v/v/v). Aqueous phytic acid hydrolysate (200 μl; 25 μg phosphate/tube). The tubes are centrifuged and 600 μl of the aqueous phase was diluted into 10 ml water.

Inositol phosphates are separated by ion-exchange chromatography using AG1-X8 in either the chloride- or formate-form. When only IP_3 levels are to be determined, the chloride-form is used, whereas the formate form is used to resolve the major inositol phosphates (IP_3, IP_2, and IP_1). For determination of just IP_3, the diluted sample is applied to the chloride-form column and the column is washed with 10 ml 30 mM HCl followed by 6 ml 90 mM HCl and the IP_3 is eluted with 3 ml 500 mM HCl. The last eluate is diluted and counted. For determination of all major inositol phosphates, the diluted sample is applied to the formate-form column and IP_1, IP_2, and IP_3 eluted sequentially by increasing concentrations of formate buffer. The eluted samples from the formate columns are rotary evaporated, the residues brought up in cocktail, and counted.

The isomeric forms of IP_3 are evaluated by HPLC. The reactions are terminated by the addition of 1 ml 0.45 M

perchloric acid and stored on ice for 10 minutes. Following centrifugation, the supernatant is adjusted to pH 7-8 with $NaHCO_3$. The extract is then applied to a Partisil SAX anion-exchange column and eluted with a linear gradient of ammonium formate. The various fractions are then desalted with Dowex followed by rotary evaporation prior to liquid scintillation counting in a Packard Tri-carb 1500 LSC.

For all inositol phosphate separation methods, appropriate controls using authentic standards are used to determine if organic polycations interfered with the separation. If so, the samples are treated with cation-exchange resin to remove the offending molecule prior to separation of inositol phosphates.

V. Modulation of Metabotropic Glutamate Receptor Activity

Modulation of metabotropic glutamate receptor activity can be used to produce different effects such as anticonvulsant effects, neuroprotectant effects, analgesic effects, cognition-enhancement effects, and muscle-relaxation effects. Each of these effects has therapeutic applications. Compounds used therapeutically should have minimal side effects at therapeutically effective doses.

The ability of a compound to modulate metabotropic glutamate activity can be assessed using electrophysiological and biochemical assays measuring one or more metabotropic glutamate activities. Examples of such assays include the electrophysiological or biochemical assessment of metabotropic glutamate receptor function in *Xenopus* oocytes expressing cloned metabotropic glutamate receptors or chimeric receptors; the electrophysiological or biochemical assessment of metabotropic glutamate receptor function in transfected cell lines (*e.g.*, CHO cells, HEK 293 cells, *etc.*) expressing cloned metabotropic glutamate receptors or chimeric receptors; the biochemical assessment of PI hydrolysis, cAMP accumulation, activation of phospholipase

C, increase in inositol phosphate, increase in intracellular calcium, mobilization of intracellular calcium, activation of phospholipase D, activation or inhibition of adenylate cyclase, increases or decreases in the formation of cyclic adenosine monophosphate (cAMP), activation of guanylate cyclase, increases in the formation of cyclic guanosine monophosphate (cGMP), activation of phospholipase A_2, and increases in arachidonic acid release; the biochemical or electrophysiological assessment of increases or decreases in the activity of voltage- and ligand-gated ion channels, in transfected cell lines expressing cloned metabotropic glutamate receptors or chimeric receptors; the biochemical assessment of PI hydrolysis, cAMP accumulation, activation of phospholipase C, increase in inositol phosphate, increase in intracellular calcium, mobilization of intracellular calcium, activation of phospholipase D, activation or inhibition of adenylate cyclase, increases or decreases in the formation of cyclic adenosine monophosphate (cAMP), activation of guanylate cyclase, increases in the formation of cyclic guanosine monophosphate (cGMP), activation of phospholipase A_2, increases in arachidonic acid release, and increases or decreases in the activity of voltage- and ligand-gated ion channels, in rat brain (*e.g.*, hippocampal, cortical, striatal, *etc.*) slices; fluorimetric measurements of cytosolic Ca^{2+} in cultured rat cerebellar granule cells; and fluorimetric measurements of cytosolic Ca^{2+} in transfected cell lines expressing cloned metabotropic glutamate receptors or chimeric receptors.

Prior to therapeutic use in a human, the compounds are preferably tested *in vivo* using animal models. Animal studies to evaluate a compound's effectiveness to treat different diseases or disorders, or exert an effect such as an analgesic effect, a cognition-enhancement effect, or a muscle-relaxation effect, can be carried out using standard techniques.

A. Treatment of Neurological Diseases and Disorders

A preferred use of the compounds and methods of the present invention is in the treatment of neurological diseases and disorders. Patients suffering from a neurological disease or disorder can be diagnosed by standard clinical methodology.

Neurological diseases or disorders include neuronal degenerative diseases, glutamate excitotoxicity, global and focal ischemic and hemorrhagic stroke, head trauma, spinal cord injury, hypoxia-induced nerve cell damage, and epilepsy. These different diseases or disorders can be further medically characterized. For example, neuronal degenerative diseases include Alzheimer's disease, Parkinson's disease, and Huntington's disease.

B. Production of Other Therapeutic Effects

Another preferred use of the present invention is in the production of other therapeutic effects, such as analgesic effects, cognition-enhancement effects, or muscle-relaxation effects. The present invention is preferably used to produce one or more of these effects in a patient in need of such treatment.

Patients in need of such treatment can be identified by standard medical techniques. For example, the production of analgesic activity can used to treat patients suffering from clinical conditions of acute and chronic pain including the following: preemptive preoperative analgesia; peripheral neuropathies such as occur with diabetes mellitus and multiple sclerosis; phantom limb pain; causalgia; neuralgias such as occur with herpes zoster; central pain such as that seen with spinal cord lesions; hyperalgesia; and allodynia.

VI. Formulation and Administration

The optimal formulation and mode of administration of compounds of the present application to a patient depend on factors known in the art such as the particular disease

or disorder, the desired effect, and the type of patient. While the compounds will typically be used to treat human patients, they may also be used to treat similar or identical diseases in other vertebrates such as other primates, farm animals such as swine, cattle and poultry, and sports animals and pets such as horses, dogs and cats.

Preferably, the therapeutically effective amount is provided as a pharmaceutical composition. A pharmacological agent or composition refers to an agent or composition in a form suitable for administration into a multicellular organism such as a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should allow the agent or composition to reach a target cell whether the target cell is present in a multicellular host or in culture. For example, pharmacological agents or compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms which prevent the agent or composition from exerting its effect.

The claimed compositions can also be formulated as pharmaceutically acceptable salts (*e.g.*, acid addition salts) and/or complexes thereof. The preparation of such salts can facilitate the pharmacological use by altering the physical-chemical characteristics of the composition without preventing the composition from exerting its physiological effect. Examples of useful alterations in physical properties include lowering the melting point to facilitate transmucosal administration and increasing the solubility to facilitate the administration of higher concentrations of the drug.

The compounds of the invention can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Co., Easton, PA, 1990.

For systemic administration, oral administration is preferred. For oral administration, the compounds are formulated into conventional oral dosage forms such as capsules, tablets and tonics.

Alternatively, injection may be used, *e.g.*, intramuscular, intravenous, intraperitoneal, subcutaneous, intrathecal, or intracerebroventricular. For injection, the compounds of the invention are formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. Alternatively, the compounds of the invention are formulated in one or more excipients (*e.g.*, propylene glycol) that are generally accepted as safe as defined by USP standards. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration may be, for example, through nasal sprays or using suppositories.

For topical administration, the compounds of the invention are formulated into ointments, salves, gels, or creams, as is generally known in the art.

VII. Examples

Examples are provided below to illustrate different aspects and embodiments of the present invention. These examples are not intended in any way to limit the disclosed invention. Rather, they illustrate methodology by which compounds of the present invention can be readily

identified by routine procedures to ensure that they have the desired activity, and the synthesis of different compounds described herein. Compounds within claimed structures can be screened to determine those with the most appropriate activities prior to their use.

Example 1: Activation of Metabotropic Glutamate Receptors Expressed in *Xenopus* Oocytes

This example describes the activation of mGluR1 using a *Xenopus* oocyte expression assay. In this example, the compound tested for activity was Compound A. Those skilled in the art will recognize that any of the compounds of the present invention, including the compounds of Figure 1, may be tested using this assay. Rat mGluR1 cDNA (Masu *et al.*, *Nature 349*:760-765, 1991) was subcloned into the pBluescript vector, linearized by NotI digestion and capped sense-strand cRNA was synthesized by T7 RNA polymerase transcription (Melton *et al.*, *Nucl. Acids Res. 12*:7035-7056, 1984). *In vitro*-transcribed RNA was concentrated by ethanol precipitation and the size and integrity of the RNA was assessed on denaturing agarose gels.

Xenopus oocytes were injected with 50 ng of cRNA encoding rat mGluR1. After 4 days incubation, two-electrode voltage clamp recording was used to monitor the endogenous Ca^{2+}-dependent Cl^- current in response to added compounds. Details of the oocyte manipulations and electrophysiological procedures are described by Racke *et al.*, *FEBS Letters 333*:132-136, 1993.

Following compound addition, oocytes were washed prior to subsequent compound additions. Uninjected oocytes served as a negative control (experiment A). In experiment B, mGluR1-expressing oocytes were challenged twice with 1 mM L-glutamate (L-Glu). In experiment C, mGluR1-expressing oocytes were challenged twice with 1 μM Compound A followed by a challenge with 1 mM L-glutamate (L-Glu).

Uninjected, control oocytes did not respond to either 1 mM glutamate or 1 μM Compound A. Application of 1 mM glutamate to oocytes injected with mGluR1 cRNA resulted in a large (> 1 μA) increase in outward chloride current typical for activation of a G-protein-coupled receptors coupling to PI hydrolysis. Subsequent addition of glutamate produced no response, indicating that the initial stimulation resulted in receptor desensitization (*i.e.*, agonist induced loss of receptor function).

Similarly, application of 1 μM Compound A to oocytes expressing mGluR1 resulted in a large increase in outward chloride current. Subsequent addition of 1 mM glutamate failed to elicit a response, which may indicate that the action of Compound A was due to activation and subsequent desensitization of the mGluR1. Taken together, these results may indicate that Compound A activates mGluR1.

Example 2: Recombinant Receptor Binding Assay

The following is one example of a rapid screening assay to obtain compounds modulating metabotropic glutamate receptor activity. In this example, the compound tested for activity was Compound A. Those skilled in the art will recognize that any of the compounds of the present invention, including the compounds of Figure 1, may be tested using this assay. The screening assay first measures the ability of compounds to bind to recombinant receptors, or receptor fragments containing the mGluR:CaR site. Compounds binding to the metabotropic glutamate receptor are then tested for their ability to modulate one or more activities at a metabotropic glutamate receptor.

In one procedure, a cDNA or gene clone encoding the metabotropic glutamate receptor from a suitable organism such as a human is obtained using standard procedures. Distinct fragments of the clone are expressed in an appropriate expression vector to produce the smallest receptor polypeptide(s) obtainable able to bind Compound

A. In this way, the polypeptide(s) containing the mGluR:CaR binding site is identified. Such experiments can be facilitated by utilizing a stably transfected mammalian cell line (*e.g.*, HEK 293 cells) expressing metabotropic glutamate receptors or portion(s) thereof.

Alternatively, the metabotropic glutamate receptor or portion(s) thereof can be chemically reacted with Compound A chemically modified so that amino acid residues of the metabotropic glutamate receptor which contact (or are adjacent to) the selected compound are modified and thereby identifiable. The fragment(s) of the metabotropic glutamate receptor containing those amino acids which are determined to interact with Compound A and are sufficient for binding to Compound A, can then be recombinantly expressed using standard techniques.

The recombinant polypeptide(s) having the desired binding properties can be bound to a solid-phase support using standard chemical procedures. This solid-phase, or affinity matrix, may then be contacted with Compound A to demonstrate that this compound can bind to the column, and to identify conditions by which the compound may be removed from the solid-phase. This procedure may then be repeated using a large library of compounds to determine those compounds which are able to bind to the affinity matrix. Bound compounds can then can be released in a manner similar to Compound A. Alternative binding and release conditions may be utilized to obtain compounds capable of binding under conditions distinct from those used for Compound A binding (*e.g.*, conditions which better mimic physiological conditions encountered especially in pathological states). Compounds binding to the mGluR:CaR site can thus be selected from a very large collection of compounds present in a liquid medium or extract.

In an alternate method, native metabotropic glutamate receptors are bound to a column or other solid phase support. Those compounds which are not competed off by reagents binding to the glutamate binding site on the

receptor can then be identified. Such compounds define alternative binding sites on the receptor. Such compounds may be structurally distinct from known compounds and may define chemical classes of agonists or antagonists which may be useful as therapeutics agents.

Example 3: Screening for Compounds Active at the mGluR:CaR Site using Radioligand Binding to Cell Membranes Containing the Metabotropic Glutamate Receptor or Chimeric Receptor

Other compounds can be identified which act at mGluR's using the procedures described in this section. A high-throughput assay is first used to screen product libraries (*e.g.*, natural product libraries and compound files) to identify compounds with activity at the mGluR:CaR site. These compounds are then utilized as chemical lead structures for a drug development program targeting the mGluR:CaR site on metabotropic glutamate receptors. Routine experiments, including animal studies can be performed to identify those compounds having the desired activities.

The following assay can be utilized as a high-throughput assay. Rat brain membranes are prepared according to the method of Williams *et al.*, *Molec. Pharmacol. 36*:575, (1989), with the following alterations: Male Sprague-Dawley rats (Harlan Laboratories) weighing 100-200 g are sacrificed by decapitation. The cortex or cerebellum from 20 rats are cleaned and dissected. The resulting brain tissue is homogenized at 4°C with a polytron homogenizer at the lowest setting in 300 ml 0.32 M sucrose containing 5 mM K-EDTA (pH 7.0). The homogenate is centrifuged for 10 min at 1,000 x *g* and the supernatant removed and centrifuged at 30,000 x *g* for 30 minutes. The resulting pellet is resuspended in 250 ml 5 mM K-EDTA (pH 7.0) stirred on ice for 15 minutes, and then centrifuged at 30,000 x *g* for 30 minutes. The pellet is resuspended in 300 ml 5 mM K-EDTA (pH 7.0) and incubated at 32°C for

30 minutes. The suspension is then centrifuged at 100,000 x *g* for 30 minutes. Membranes are washed by resuspension in 500 ml 5 mM K-EDTA (pH 7.0), incubated at 32°C for 30 minutes, and centrifuged at 100,000 x *g* for 30 minutes. The wash procedure, including the 30-minute incubation, is repeated. The final pellet is resuspended in 60 ml 5 mM K-EDTA (pH 7.0) and stored in aliquots at -80°C. Preparation of cell membranes from recombinant cells expressing individual cloned metabotropic glutamate receptors or chimeric receptors are similarly prepared with minor modifications known to those of ordinary skill in the art.

To perform a binding assay with [^{3}H]Compound A, aliquots of SPMs (synaptic plasma membranes) or membranes from recombinant cells are thawed, resuspended in 30 ml of 30 mM EPPS/1 mM K-EDTA, pH 7.0, and centrifuged at 100,000 x *g* for 30 minutes. SPMs are resuspended in buffer A (30 mM EPPS/1 mM K-EDTA, pH 7.0). The [^{3}H] lead compound is added to this reaction mixture. Binding assays are carried out in polypropylene test tubes. The final incubation volume is 500 µl. Nonspecific binding is determined in the presence of 100 µM nonradioactive lead compound. Duplicate samples are incubated at 0°C for 1 hour. Assays are terminated by adding 3 ml of ice-cold buffer A, followed by filtration over glass-fiber filters (Schleicher & Schuell No. 30) that are presoaked in 0.33% polyethyleneimine (PEI). The filters are washed with another 3 x 3 ml of buffer A, and radioactivity is determined by scintillation counting at an efficiency of 35-40% for ^{3}H.

In order to validate the above assay, the following experiments can also be performed:

(a) The amount of nonspecific binding of the [^{3}H] lead compound to the filters is determined by passing 500 µl of buffer A containing various concentrations of [^{3}H] lead compound through the presoaked glass-fiber filters. The filters are washed with another 4 x 3 ml of buffer A, and

radioactivity bound to the filters is determined by scintillation counting at an efficiency of 35-40% for ^3H.

(b) A saturation curve is constructed by resuspending membranes in buffer A. The assay buffer (500 µl) contains 60 µg of protein. Concentrations of [^3H] lead compound are used, ranging from 1.0 nM to 400 µM in half-log units. A saturation curve is constructed from the data, and an apparent K_D value and B_{max} value determined by Scatchard analysis (Scatchard, *Ann. N.Y. Acad. Sci. 51*: 660, 1949). The cooperativity of binding of the [^3H] lead compound is determined by the construction of a Hill plot (Hill, *J. Physiol. 40*:190, 1910).

(c) The dependence of binding on protein (receptor) concentration is determined by resuspending membranes in buffer A. The assay buffer (500 µl) contains a concentration of [^3H] lead compound equal to its K_D value and increasing concentrations of protein. The specific binding of [^3H] lead compound should be linearly related to the amount of protein (receptor) present.

(d) The time-course of ligand-receptor binding is determined by resuspending membranes in buffer A. The assay buffer (500 µl) contains a concentration of [^3H] lead compound equal to its K_D value and 100 µg of protein. Duplicate samples are incubated at 0°C for varying lengths of time; the time at which equilibrium is reached is determined, and this time point is routinely used in all subsequent assays.

(e) The pharmacology of the binding site can be analyzed by competition experiments. In such experiments, the concentration of [^3H] lead compound and the amount of protein are kept constant, while the concentration of test (competing) drug is varied. This assay allows for the determination of an IC_{50} and an apparent K_D for the competing drug (Cheng and Prusoff, *J. Biochem. Pharmacol. 22*:3099, 1973). The cooperativity of binding of the competing drug is determined by Hill plot analysis.

Specific binding of the [^{3}H] lead compound represents binding to the mGluR:CaR site on metabotropic glutamate receptors. As such, analogs of the lead compound should compete with the binding of [^{3}H] lead compound in a competitive fashion, and their potencies in this assay should correlate with their potencies in a functional assay of metabotropic glutamate receptor activity (*e.g.*, electrophysiological assessment of the activity of cloned metabotropic glutamate receptors expressed in *Xenopus* oocytes). Conversely, compounds which have activity at the known glutamate binding site on metabotropic glutamate receptors should not displace [^{3}H] lead compound binding in a competitive manner. Rather, complex allosteric modulation of [^{3}H] lead compound binding, indicative of noncompetitive interactions, might occur.

(f) Studies estimating the dissociation kinetics are performed by measuring the binding of [^{3}H] lead compound after it is allowed to come to equilibrium (see (d) above), and a large excess of nonradioactive competing drug is added to the reaction mixture. Binding of the [^{3}H] lead compound is then assayed at various time intervals. With this assay, the association and dissociation rates of binding of the [^{3}H] lead compound are determined (Titeler, *Multiple Dopamine Receptors: Receptor Binding Studies in Dopamine Pharmacology*. Marcel Dekker, Inc., New York, 1983). Additional experiments involve varying the reaction temperature (0°C to 37°C) in order to understand the temperature dependence of this parameter.

Those in the art will appreciate that membranes may be prepared from recombinant cells expressing individual metabotropic glutamate receptors, calcium receptors or chimeric receptors and may be used in place of rat cortex membranes in the above example with minor modifications known to those in the art. One example is provided in O'Hara et al., Neuron, 11:41, (1993) hereby incorporated by reference herein.

Example 4: Chimeric Receptor Assay

Chimeric receptors may be constructed according to the methods described in the copending U.S. application filed by Fuller et al., on July 26, 1995, entitled "Chimeric Receptors and Methods for Identifying Compounds Active at Metabotropic Glutamate Receptors and the Use of Such Compounds in the Treatment of Neurological Disorders and Diseases", hereby incorporated by referenced herein. These chimeric receptors allow for a highly sensitive, high throughput assay for compounds which modulate mGluRs. The chimeric receptor used may consist of, for example, the ECD and 7TMD of an mGluR, and the cytoplasmic tail of a CaR.

a. *In vitro* transcription of RNA

RNA transcripts encoding the chimeric receptors may be produced by enzymatic transcription from plasmid templates using T7 polymerase supplied with the mMessage mMachine ™(Ambion). Each plasmid is then treated with a restriction enzyme to make a single cut distal to the 3' end of the cDNA insert to linearize the template. This DNA is incubated with T7 RNA polymerase in the presence of GpppG cap nucleotide, rATP, rCTP, rUTP and rGTP. The synthetic RNA transcript is purified by DNase treatment of the reaction mix and subsequent alcohol precipitations. RNA may be quantitiated by absorbance spectroscopy (OD_{260}) and visualized on an ethidium stained 1.2% formaldehyde gel.

b: Functional expression in oocytes

Oocytes suitable for injection are obtained from adult female *Xenopus laevis* toads using procedures described in C. J. Marcus-Sekura and M. J. M. Hitchcock, *Methods in Enzymology*, Vol. 152 (1987). Pieces of ovarian lobe are incubated for 30 minutes in Ca^{2+}-free Modified Barths Saline (MBS) containing 1.5 mg/ml collagenase type IA (Worthington). Subsequently, 5 ng of RNA transcript

prepared as described in Example a, is injected into each oocyte. Following injection, oocytes are incubated at 16°C in MBS containing 0.5 mM $CaCl_2$ for 2-7 days prior to electrophysiological examination.

The ability of each chimeric receptor to function is determined by voltage-recording of current-passing electrodes across the oocyte membrane in response to glutamate and calcium receptor agonists. Oocytes are voltage clamped at a holding potential of -60 mV with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) using standard two electrode voltage-clamp techniques . Currents are recorded on a chart recorder. The standard control saline is MBS containing 0.3 mM $CaCl_2$ and 0.8 $MgCl_2$. Test compounds are applied by superfusion at a flow rate of about 5 ml/min. All experiments are done at room temperature. The holding current should be stable in a given oocyte and may vary between +10 to -200 nA for different oocytes. Activation of I_{Cl} in response to activation of receptors and subsequent increases in intracellular Ca2+ ($[Ca]_{in}$) may be quantified by measuring the peak inward current stimulated by agonist or drug, relative to the holding current at -60 mV.

c: Transfection and Growth of HEK293 Recombinant Cells Expressing Chimeric Receptors

The DNA encoding chimeric receptors, as described in this example may be inserted into functional expression vectors and used to transfect cell lines, as described in copending U.S. application filed by Fuller et al., on July 26, 1995, entitled "Chimeric Receptors and Methods for Identifying Compounds Active at Metabotropic Glutamate Receptors and the Use of Such Compounds in the Treatment of Neurological Disorders and Diseases," hereby incorporated by reference herein. Human embryonic kidney cells (293, ATCC, CRL 1573) are grown in a routine manner. Cells are plated in 10 cm cell-culture plates in Dulbecco's modified Eagle's medium (D-MEM) containing 10

% fetal calf serum (FCS) and 1 X Penicillin-Streptomycin (PS, Life Technologies) so that they are ~70% confluent after an overnight incubation. To prepare DNA for transfection, plasmid DNA comprising DNA encoding the chimeric receptor is precipitated with ethanol, rinsed and resuspended in sterile water at a concentration of 1 ug/ul. Fourteen micrograms of DNA is incubated with the liposome formulation LipofectAMINE™ (Life Technologies) for 20 minutes in serum-free Opti-MEM® (Life Technologies). After the room temperature incubation, 6.8 mls of Opti-MEM® is added to the transfection mix. This solution is added to the cells which have been rinsed with 2X 5 ml washes of serum-free Opti-MEM®. The cells and transfection mix are incubated at 37°C for 5 hours at which time more media and fetal bovine serum are added to bring the serum concentration to 10 %. After an overnight incubation the media is changed back to D-MEM with 10% FCS and 1 x PS. After an additional 24 h incubation, cells are detached with trypsin and replated in media containing 200 ug/ml hygromycin (Boerhringer Mannheim). Those cells which survive hygromycin selection contain the chimeric receptor encoding plasmid which also contains the hygromycin resistance gene. Individual clones are recovered and propagated using standard tissue-culture techniques.

d. Measurement of Intracellular Calcium

Intracellular calcium is quantified using the fluorescent calcium indicator, fura-2, as described in Parks et al., 1989, hereby incorporated by reference herein. Stably transfected cells containing the chimeric plasmid are loaded with 2 μM fura-2 acetoxymethylester by incubation for 20-30 minutes at 37°C in SPF-PCB (126 mM NaCl, 5mM KCl, 1mM $MgCl_2$, 20 mM HEPES, pH 7.4), containing 1.25 mM $CaCl_2$, 1 mg/ml glucose, 0.5% BSA[1]. The cells are then washed 1 to 2 times in SPF-PCB containing 0.5 mM $CaCl_2$, 0.5% BSA and resuspended to a density of 4 to 5

million cells/ml and kept at 22°C in a plastic beaker. The cells are diluted fivefold into a quartz cuvette with BSA-free 37°C SPF-PCB to achieve a final BSA concentration of 0.1% (1.2 ml of 37°C BSA-free SPF-PCB + 0.3 ml cell suspension). Measurements of fluorescence are performed at 37°C with constant stirring using a custom-built spectrofluorimeter (Biomedical Instrumentation Group, University of Pennsylvania). Excitation and emission wavelengths are 340 and 510 nm, respectively. Digitonin (Sigma, St. Louis, MO; catalog # D 5628; 50 μg/ml, final) is added to obtain F_{max} , and the apparent F_{min} is determined by adding EGTA (10 mM, final) and Tris base (pH ~ 10, final). Concentrations of intracellular Ca^{2+} are calculated using a dissociation constant (Kd) of 224 nM and the equation:

$$[Ca^{2+}]_i = (F - F_{min}/F_{max} - F) \times Kd$$

Example 5: Preparation of Compound A

Compound A (N-[3-(2-chlorophenyl)propyl]-(R)-α-methyl-3-methoxybenzylamine) was prepared as described in Nemeth *et al*, WO93/04373 *supra*. In a 250-ml round-bottom flask, 10.0 g (100 mmoles) 3'-methoxyacetophenone and 13.5 g (100 mmoles) 3-(2-chlorophenyl)propylamine were mixed and treated with 125 mmoles (35.5 g) titanium(IV) isopropoxide. The reaction mixture was stirred 30 minutes at room temperature under a nitrogen atmosphere. After this time 6.3 g (100 mmoles) sodium cyanoborohydride in 100 ml ethanol was added dropwise over the course of 2 minutes. The reaction was stirred at room temperature under nitrogen for 16 hours. After this time the reaction mixture was transferred to a 2 L separatory funnel with 1.5 L diethyl ether and 0.5 L water. The phases were equilibrated and the ether layer removed. The remaining aqueous phase was thoroughly extracted with four 1 L portions of ether. The washes were combined, dried over

anhydrous potassium carbonate and reduced to a clear, light amber oil.

TLC analysis of this material on silica gel using chloroform-methanol-isopropylamine (100:5:1) showed product at R_f 0.65 with traces of the two starting materials at R_f 0.99 (3'-methoxy acetophenone) and R_fh 0.0 (3-phenylpropylamine).

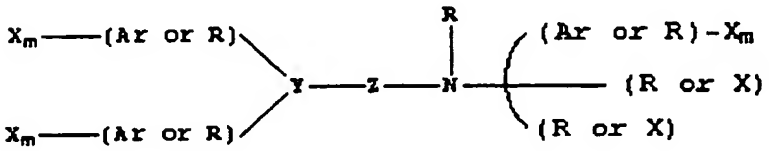
The reaction mixture was chromatographed through silica (48 x 4.6 cm) using a gradient of chloroform-methanol-isopropylamine (99:1:0.1) to (90:10:0.1) which yielded purified Compound A. This material was dissolved in hexane-isopropanol (99:1) containing 0.1% diethylamine to yield a solution with a concentration of 50 mg/ml. Chiral resolution was accomplished by chromatography of 4 ml of this solution (200 mg, maximum to achieve separation) through ChiralCel OD (25 x 2 cm) using 0.7% isopropylamine, 0.07% diethylamine in hexane at 10 ml/min, monitoring optical density at 260 nm. Each optical isomer (free base) was converted to the corresponding hydrochloride salt by dissolving 3 g of the free base in 100 ml ethanol and treating it with 100 ml water containing 10 molar equivalents HCl. Lyophilization of this solution yielded a white solid.

The above procedure can be modified to increase yield. It was found that allowing the mixture of 3'-methoxyacetophenone, 3-(2-chlorophenyl)propylamine and titanium(IV) isopropoxide to stir for 5 hours prior to treatment with $NaCNBH_3$/EtOH resulted in significantly greater yield (98%).

Other embodiments are within the following claims.

Claims

1. A method for modulating metabotropic glutamate receptor activity comprising the step of contacting said receptor with a compound having the chemical structure:



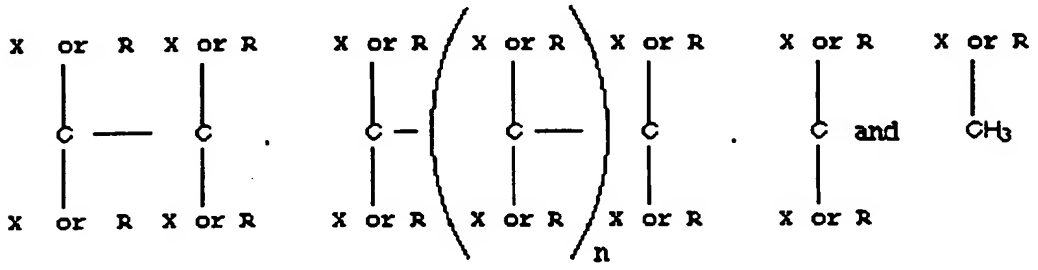
wherein each X independently is selected from the group consisting of H, CH_3, CH_3O, CH_3CH_2O, methylene dioxy, Br, Cl, F, CF_3, CHF_2, CH_2F, CF_3O, CH_3S, OH, CH_2OH, $CONH_2$, CN, NO_2, CH_3CH_2, propyl, isopropyl, butyl, isobutyl, t-butyl, and acetoxy;

Ar is a hydrophobic entity;

each R independently is selected from the group consisting of hydrogen, methyl, ethyl, propyl, isopropyl, butyl, allyl, isobutyl, t-butyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, indenyl, indanyl, dihydroindolyl, thiodihydroindolyl, 2-, 3-, or 4- piperid(in)yl;

Y is selected from the group consisting of CH, nitrogen and an unsaturated carbon; and

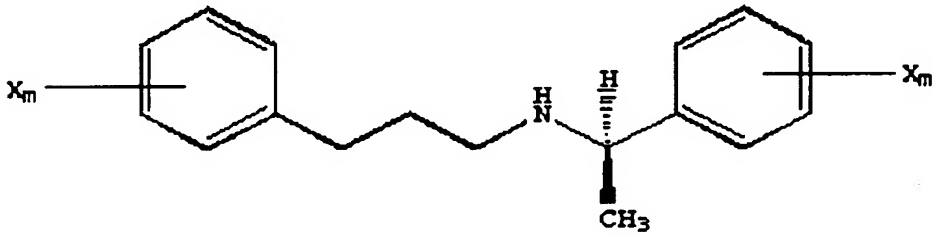
Z is selected from the group consisting of oxygen, nitrogen, sulfur,



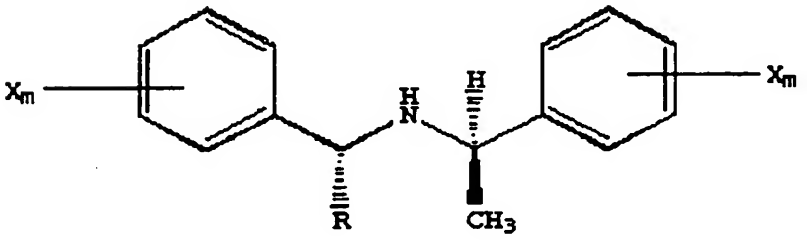
wherein each n is independently between 1 and 4 inclusive, and each m is independently between 0 and 5 inclusive; and

wherein said compound increases or decreases one or more activities of said receptor *in vivo* or *in vitro*.

2. The method of claim 1 wherein said compound has the structure:



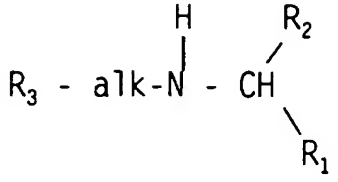
or



wherein each X is independently selected from the group consisting of Cl, F, CF_3, CH_3, isopropyl, CH_3O, CH_3S, CF_3O, aliphatic ring and aromatic ring; and

R is selected from the group consisting of H, CH_3, ethyl, and isopropyl.

3. The method of claim 1, wherein said compound has the structure:



wherein alk is straight- or branched-chain alkylene of from 0 to 6 carbon atoms;

R_1 is lower alkyl of from 1 to 3 carbon atoms or lower haloalkyl of from 1 to 3 carbon atoms substituted with from 1 to 7 halogen atoms; and

R_2 and R_3 are independently selected carbocyclic aryl or cycloalkyl groups, either monocyclic or bicyclic, having 5- to 7-membered rings optionally substituted with 1 to 5 substituents independently selected from lower alkyl of 1 to 3 carbon atoms, lower haloalkyl of 1 to 3 carbon atoms substituted with 1 to 7 halogen atoms, lower alkoxy of 1 to 3 carbon atoms, halogen, nitro, amino, alkylamino, amido, lower alkylamido of 1 to 3 carbon atoms, cyano, hydroxy, acyl of 2 to 4 carbon atoms, lower hydroxyalkyl of 1 to 3 carbon atoms or lower thioalkyl of 1 to 3 carbon atoms.

4. The method of claim 1, wherein said activities are selected from the group consisting of increase in phosphoinositide hydrolysis, activation of phospholipase C, increase in inositol phosphate, increase in intracellular calcium, mobilization of intracellular calcium, activation of adenylate cyclase, inhibition of adenylate cyclase, increase in cAMP formation, decrease in cAMP formation, activation of guanylate cyclase, increase in cGMP formation, activation of phospholipase A_2, increase in the activity of voltage-gated ion channels, decrease in the activity of voltage-gated ion channels, increase in the activity of ligand-gated ion channels, decrease in the activity of ligand-gated ion channels, activation of phospholipase D, and increase in arachidonic acid formation.

5. The method of claim 1, wherein said compound is Compound A.

6. A method for modulating metabotropic glutamate receptor activity comprising the step of contacting said receptor with a compound which modulates metabotropic glutamate receptor activity.

7. The method of claim 6 wherein said compound also modulates calcium receptor activity.

8. The method of claim 6 wherein said compound binds to a calcium receptor at a calcium receptor compound binding site which is distinct from the calcium binding site.

9. The method of claim 6 wherein said compound inhibits binding of a molecule to a calcium receptor compound binding site which is distinct from the calcium binding site.

10. The method of claim 6 wherein said compound binds to the 7 transmembrane domain of a calcium receptor.

11. The method of claim 6 wherein said compound binds to the 7 transmembrane domain of a metabotropic glutamate receptor.

12. The method of claim 6 wherein said compound binds to a metabotropic glutamate receptor binding site that is distinct from the glutamate binding site.

13. The method of claim 12 wherein said metabotropic glutamate receptor binding site is related to a compound binding site on a calcium receptor.

14. A method for modulating metabotropic glutamate receptor activity comprising the step of contacting said receptor with a compound which modulates the activity of said mGluR, wherein said compound binds to said receptor and inhibits binding of a compound of claim 1 to said receptor.

15. A method for treating a patient having a neurological disease or disorder, comprising the step of administering to said patient a therapeutically effective amount of a compound which modulates metabotropic glutamate receptor activity, wherein said compound binds to a metabotropic glutamate receptor at a site that is distinct from the glutamate binding site.

16. The method of claim 15 wherein said compound also modulates calcium receptor activity.

17. The method of claim 15 wherein said compound binds to a calcium receptor at a calcium receptor compound binding site which is distinct from the calcium binding site.

18. The method of claim 15 wherein said compound inhibits binding of a molecule to a calcium receptor compound binding site which is distinct from the calcium binding site.

19. The method of claim 15 wherein said compound binds to the 7 transmembrane domain of a glutamate receptor.

20. The method of claim 15, wherein said metabotropic glutamate receptor compound binding site is related to a compound binding site on a calcium receptor.

21. The method of claim 15 wherein said compound binds to said receptor and inhibits the binding of a compound of claim 1 to said receptor.

22. The method of claim 15, wherein said neurological disease or disorder is selected from the group consisting of: neurodegenerative diseases, glutamate excitotoxicity, global and focal ischemic and hemorrhagic stroke, head trauma, spinal cord injury, hypoxia-induced nerve cell damage, and epilepsy.

23. The method of claim 15, wherein said neurological disease or disorder is selected from the group consisting of: Alzheimer's disease, Parkinson's disease and Huntington's disease.

24. The method of claim 15, wherein said composition modulates one or more activities of an mGluR1 receptor.

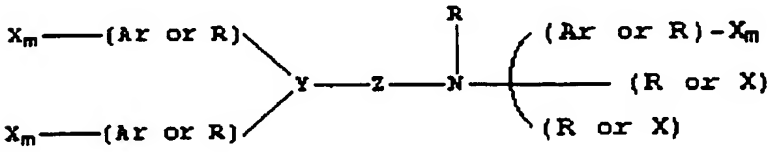
25. A method for producing an analgesic effect in a patient in need of such treatment comprising the step of administering to said patient a therapeutically effective amount of a compound which modulates metabotropic glutamate receptor activity thereby producing said

analgesic effect, wherein said compound binds to said metabotropic glutamate receptor at a site distinct from the glutamate binding site.

26. A method for enhancing cognitive abilities in a patient in need of such treatment comprising the step of administering to said patient a therapeutically effective amount of a compound which modulates metabotropic glutamate receptor activity thereby enhancing cognitive abilities, wherein said compound binds to said metabotropic glutamate receptor at a site distinct from the glutamate binding site.

27. A method for reducing muscle tension in a patient in need of such treatment comprising the step of administering to said patient a therapeutically effective amount of a compound which modulates metabotropic glutamate receptor activity thereby reducing muscle tension, wherein said compound binds to said metabotropic glutamate receptor at a site distinct from the glutamate binding site.

28. A method for treating a patient having a neurological disease or disorder, comprising the step of administering to said patient a therapeutically effective amount of a compound having the chemical structure:

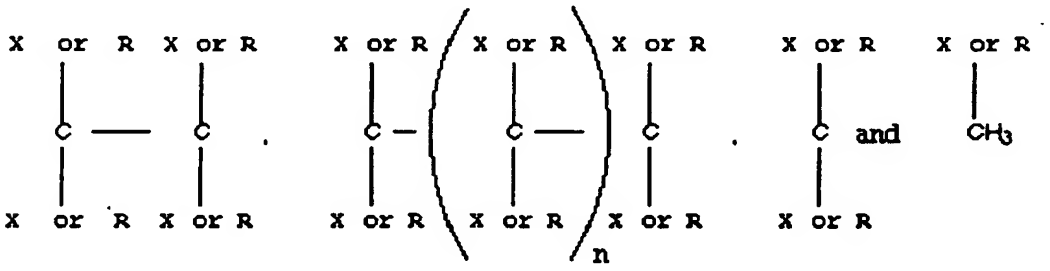


wherein each X independently is selected from the group consisting of H, CH_3, CH_3O, CH_3CH_2O, methylene dioxy, Br, Cl, F, CF_3, CHF_2, CH_2F, CF_3O, CH_3S, OH, CH_2OH, $CONH_2$, CN, NO_2, CH_3CH_2, propyl, isopropyl, butyl, isobutyl, t-butyl, and acetoxy;

Ar is a hydrophobic entity;

each R independently is selected from the group consisting of hydrogen, methyl, ethyl, propyl, isopropyl, butyl, allyl, isobutyl, t-butyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, indenyl, indanyl, dihydroindolyl, thiodihydroindolyl, 2-, 3-, or 4- piperid(in)yl;

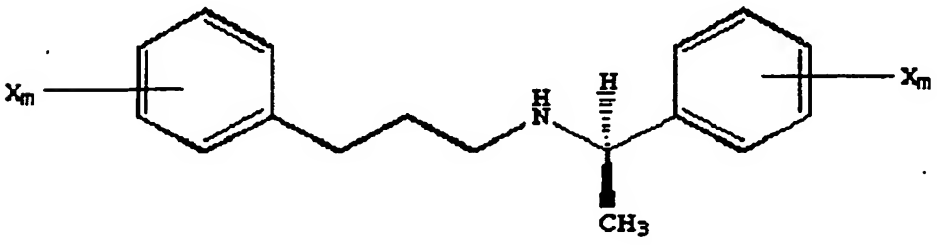
Y is selected from the group consisting of CH, nitrogen and an unsaturated carbon; and Z is selected from the group consisting of oxygen, nitrogen, sulfur,



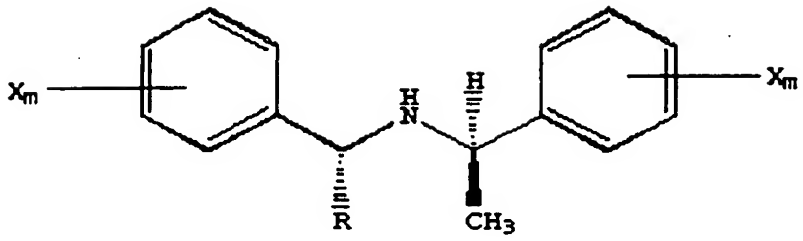
wherein each n is independently between 1 and 4 inclusive, and each m is independently between 0 and 5 inclusive; and

wherein said compound increases or decreases one or more activities of a metabotropic glutamate receptor *in vivo* or *in vitro*.

29. The method of claim 28, wherein said compound has the structure:



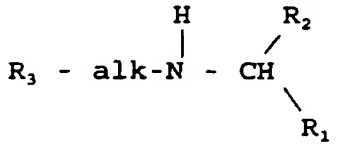
or



wherein each X is independently selected from the group consisting of Cl, F, CF_3, CH_3, isopropyl, CH_3O, CH_3S, CF_3O, aliphatic ring and aromatic ring;

and R is selected from the group consisting of H, CH_3, ethyl, and isopropyl.

30. The method of claim 28, wherein said compound has the structure:



wherein alk is straight or branched chain alkylene of from 0 to 6 carbon atoms; R_1 is lower alkyl of from 1 to 3 carbon atoms or lower haloalkyl of from 1 to 3 carbon atoms substituted with from 1 to 7 halogen atoms; R_2 and R_3 are independently selected carbocyclic aryl or cycloalkyl groups, either monocyclic or bicyclic, having 5- to 7-membered rings optionally substituted with 1 to 5 substituents independently selected from lower alkyl of 1 to 3 carbon atoms, lower haloalkyl of 1 to 3 carbon atoms substituted with 1 to 7 halogen atoms, lower alkoxy of 1 to 3 carbon atoms, halogen, nitro, amino, alkylamino, amido, lower alkylamido of 1 to 3 carbon atoms, cyano,

hydroxy, acyl of 2 to 4 carbon atoms, lower hydroxyalkyl of 1 to 3 carbon atoms or lower thioalkyl of 1 to 3 carbon atoms.

31. The method of claim 28, wherein said activities are selected from the group consisting of increase in phosphoinositide hydrolysis, activation of phospholipase C, increase in inositol phosphate, increase in intracellular calcium, mobilization of intracellular calcium, activation of adenylate cyclase, inhibition of adenylate cyclase, increase in cAMP formation, decrease in cAMP formation, activation of guanylate cyclase, increase in cGMP formation, activation of phospholipase A_2, increase in the activity of voltage-gated ion channels, decrease in the activity of voltage-gated ion channels, increase in the activity of ligand-gated ion channels, decrease in the activity of ligand-gated ion channels, activation of phospholipase D, and increase in arachidonic acid formation.

32. The method of claim 28, wherein said compound also binds to a calcium receptor.

33. The method of claim 28, wherein said compound is Compound A.

34. A method for identifying a compound able to modulate a metabotropic glutamate receptor activity comprising the steps of:

a) identifying compounds which modulate one or more activities of a calcium receptor,

b) identifying those compounds of step a) which modulate one or more activities of a metabotropic glutamate receptor.

35. A method for identifying a compound able to modulate a metabotropic glutamate receptor comprising the steps of:

a) identifying compounds which bind to a calcium receptor,

b) identifying those compounds of step a) which modulate one or more activities of a metabotropic glutamate receptor.

36. The method of claim 35 wherein the compounds identified in step a) are those compounds which bind to the 7 transmembrane domain of a calcium receptor.

37. A method for screening for a test compound able to modulate a metabotropic glutamate receptor comprising the steps of:

a) providing to said receptor a compound which binds to a binding site on a metabotropic glutamate receptor wherein said binding site is distinct from the glutamate binding site,

b) comparing the ability of said compound and said test compound to bind to said metabotropic glutamate receptor, and

c) measuring the ability of said test compound which is able to bind to said metabotropic glutamate receptor to modulate metabotropic glutamate receptor activity.

38. The method of claim 37 wherein said compound also binds to a calcium receptor.

39. The method of claim 38 wherein said compound binds to the 7 transmembrane domain of a calcium receptor.

40. The method of claim 37 wherein said test compound inhibits the binding of a compound of claim 1 to said metabotropic glutamate receptor.

41. A method for identifying a compound able to modulate a metabotropic glutamate receptor comprising the steps of:

a) identifying a first compound which binds to a calcium receptor at a compound binding site distinct from the calcium binding site,

b) identifying second compounds which inhibit the binding of said first compound to said calcium receptor by binding to said calcium receptor at a compound binding site distinct from the calcium binding site, and

c) identifying those first and second compounds of steps a) and b) which modulate one or more activities of a metabotropic glutamate receptor.

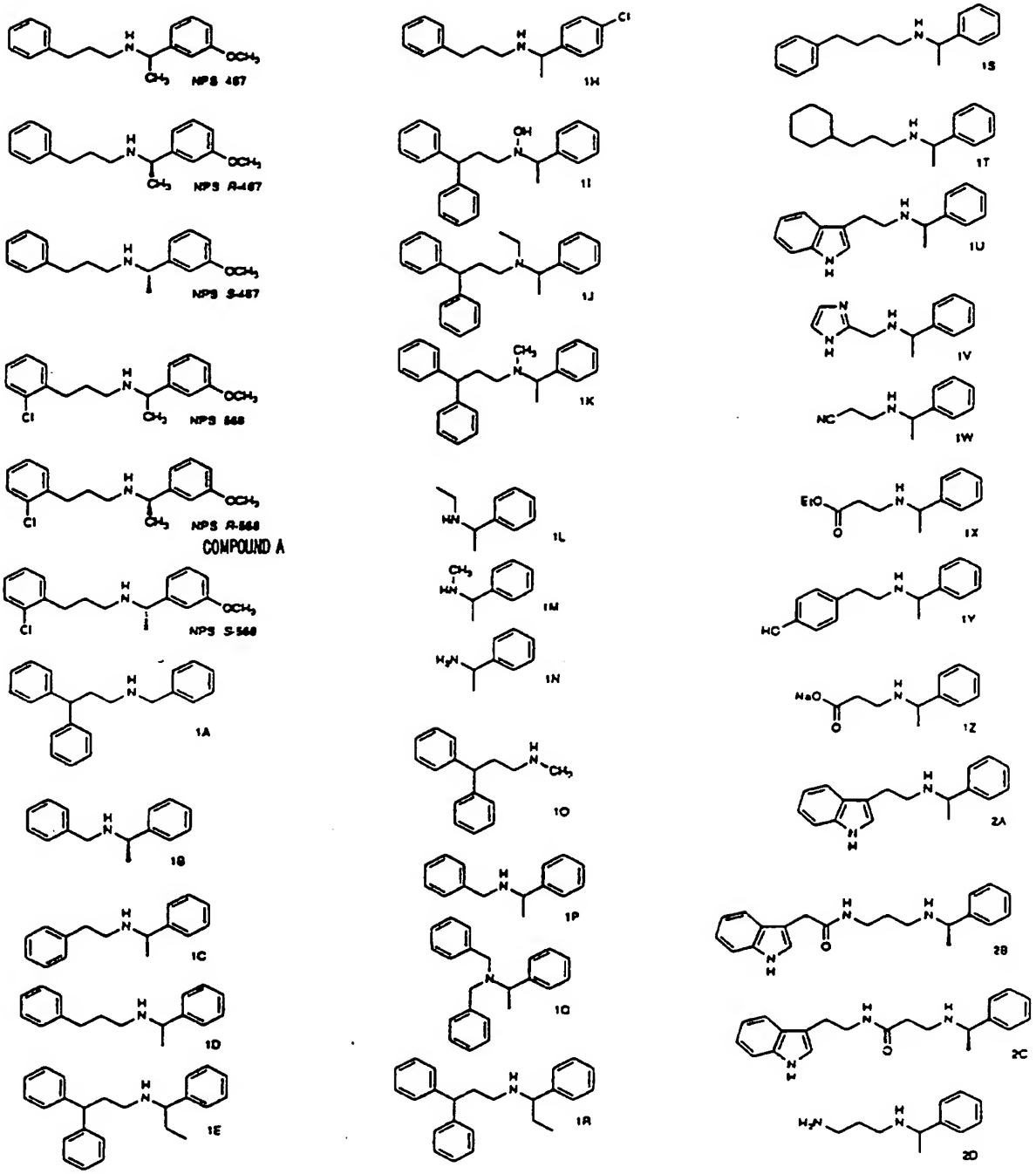
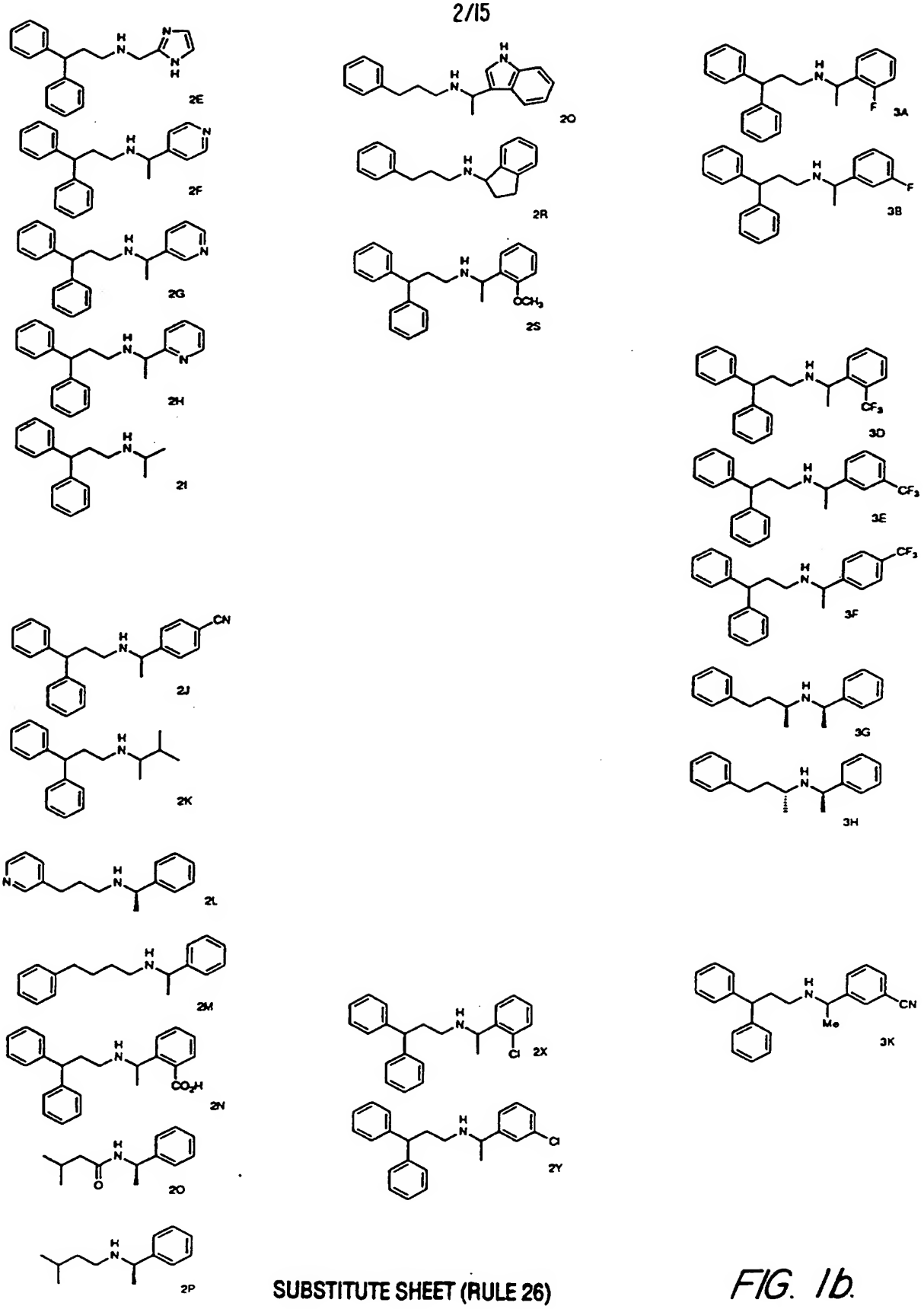

NPS 467
NPS R-467
NPS S-467
NPS 568
NPS R-568
COMPOUND A
NPS S-568
1A
1B
1C
1D
1E
1H
1I
1J
1K
1L
1M
1N
1O
1P
1Q
1R
1S
1T
1U
1V
1W
1X
1Y
1Z
2A
2B
2C
2D

FIG. 1a.


2E
2F
2G
2H
2I
2J
2K
2L
2M
2N
2O
2P
2Q
2R
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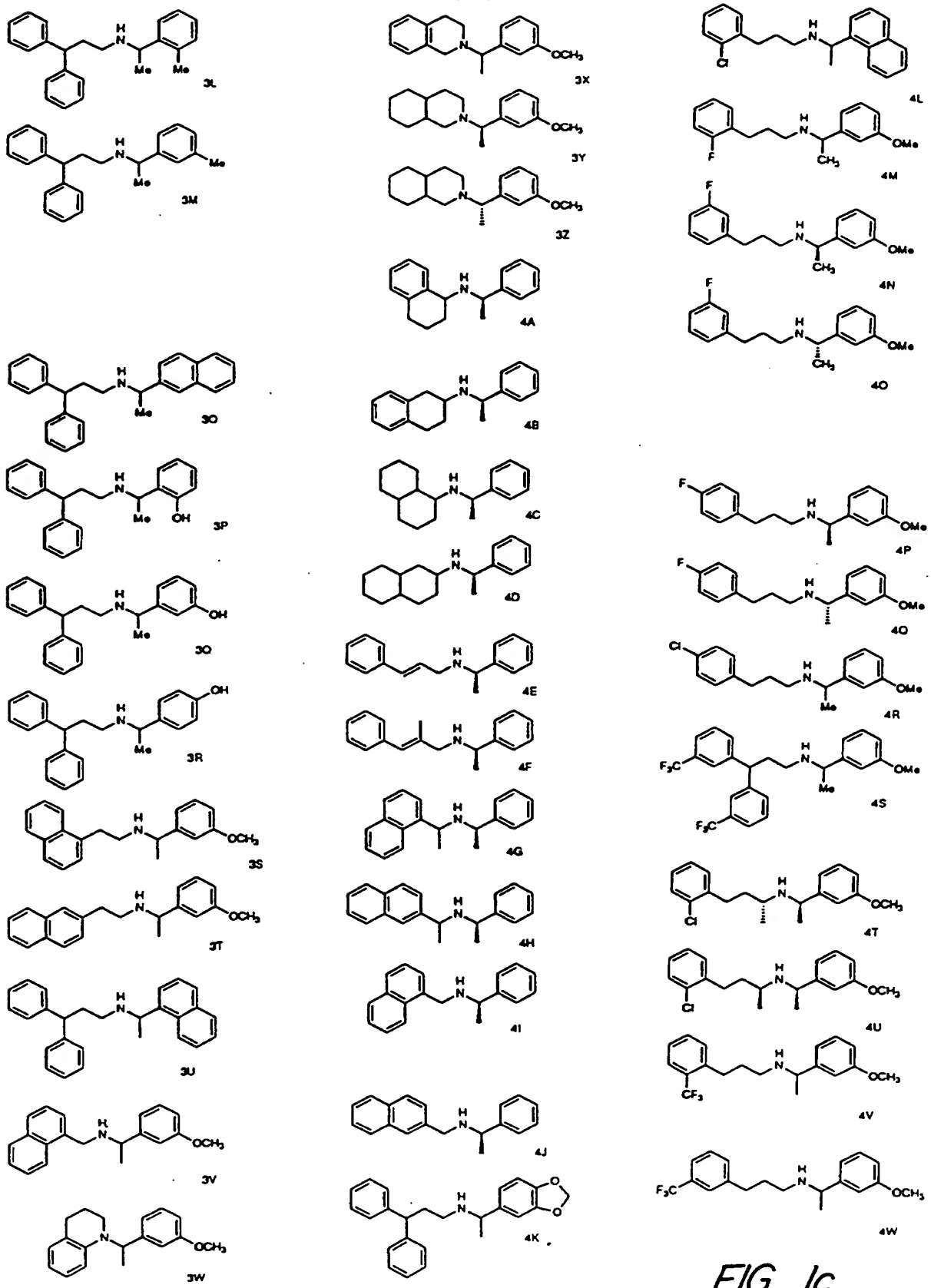
2X
2Y
3A
3B
3D
3E
3F
3G
3H
3K

FIG. 1b.


3L
3M
3O
3P
3Q
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3S
3T
3U
3V
3W
3X
3Y
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4B
4C
4D
4E
4F
4G
4H
4I
4J
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4L
4M
4N
4O
4P
4Q
4R
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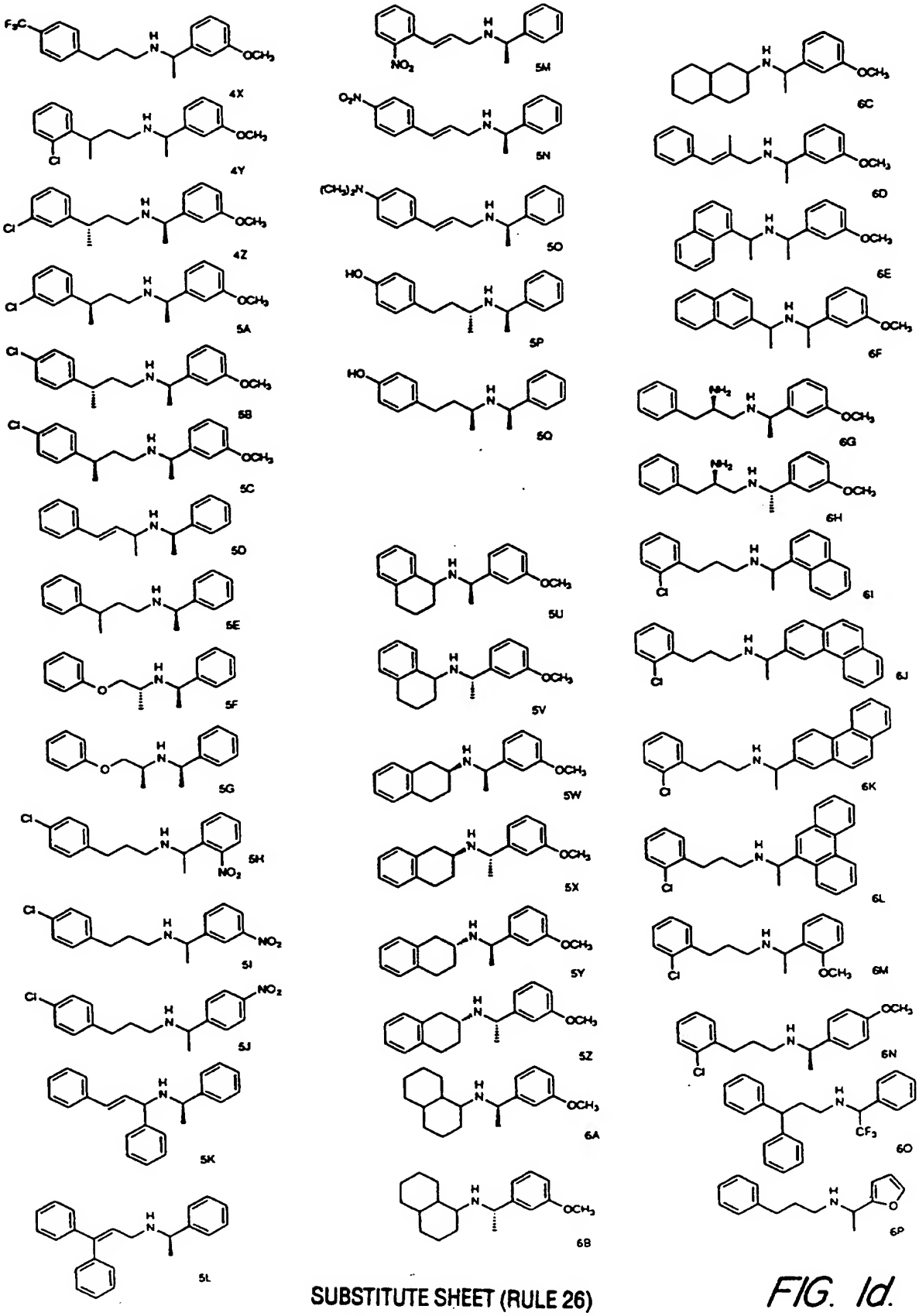
4T
4U
4V
4W

FIG. 1c.


4X
4Y
4Z
5A
5B
5C
5D
5E
5F
5G
5H
5I
5J
5K
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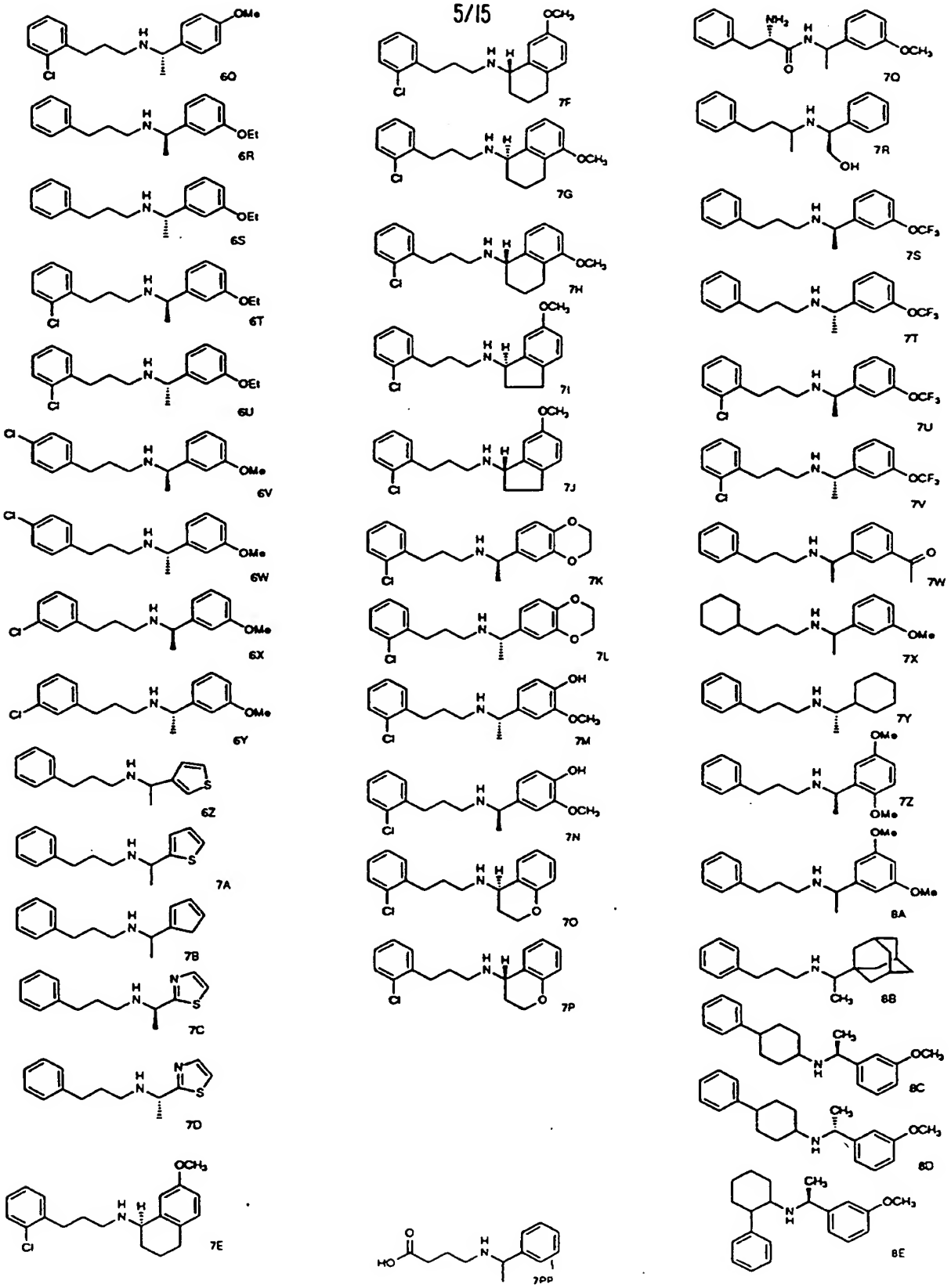
5M
5N
5O
5P
5Q
5U
5V
5W
5X
5Y
5Z
6A
6B
6C
6D
6E
6F
6G
6H
6I
6J
6K
6L
6M
6N
6O
6P

FIG. Id.


6Q
6R
6S
6T
6U
6V
6W
6X
6Y
6Z
7A
7B
7C
7D
7E
7F
7G
7H
7I
7J
7K
7L
7M
7N
7O
7P
7PP
7Q
7R
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7V
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7X
7Y
7Z
8A
8B
8C
8D
8E

FIG. 1e.

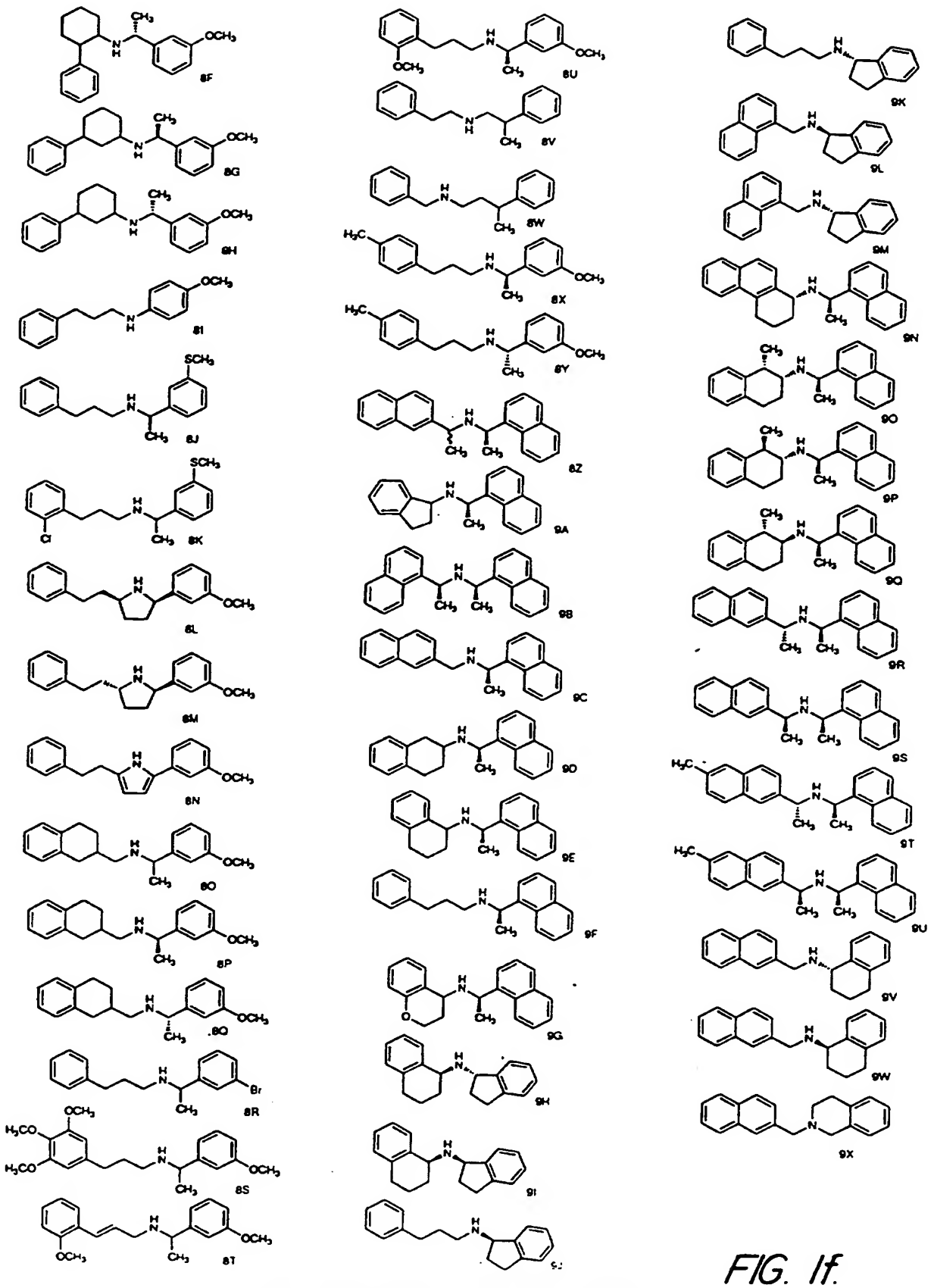
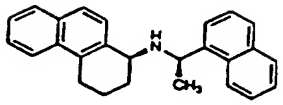
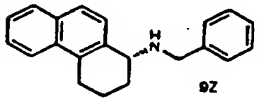


FIG. 1f.

FIG. 1g.


9Y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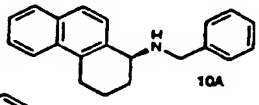
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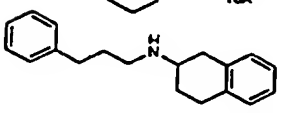
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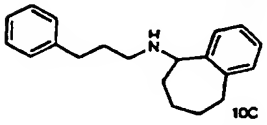
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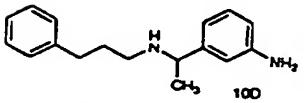
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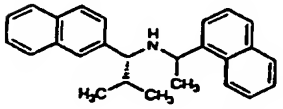
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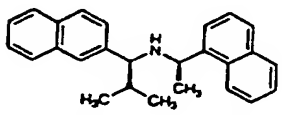
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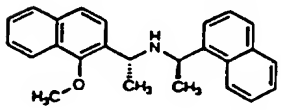
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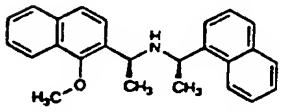
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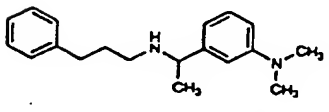
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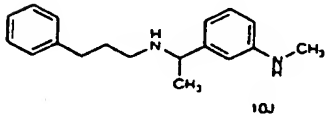
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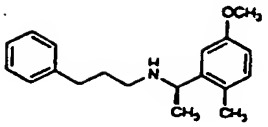
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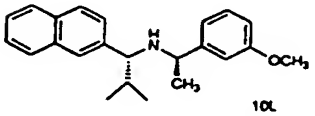
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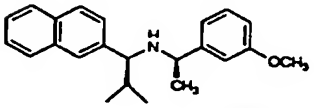
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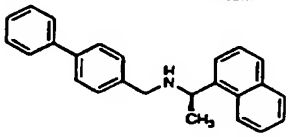
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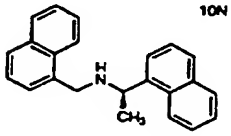
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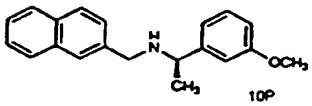
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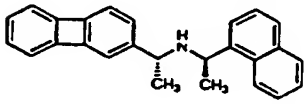
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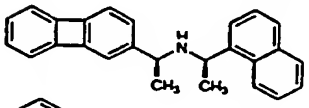
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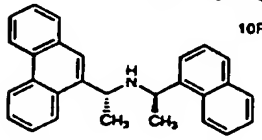
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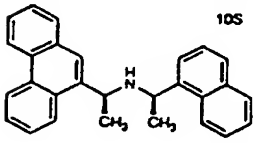
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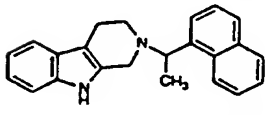
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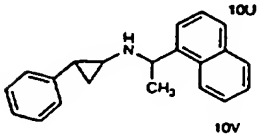
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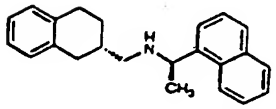
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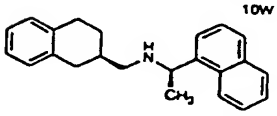
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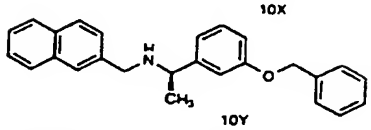
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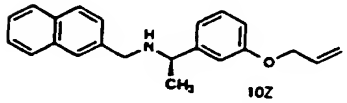
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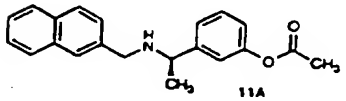
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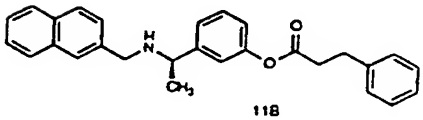
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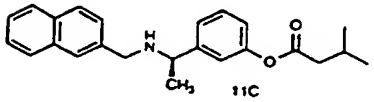
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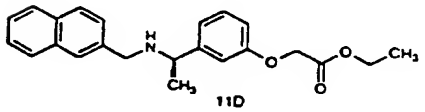
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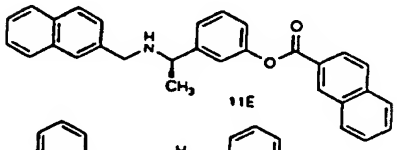
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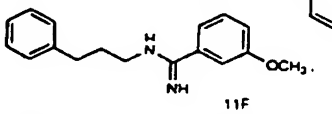
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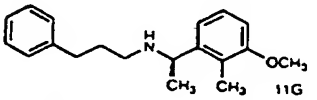
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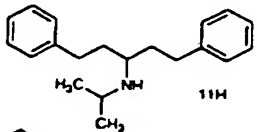
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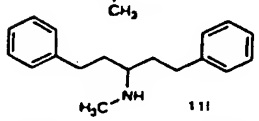
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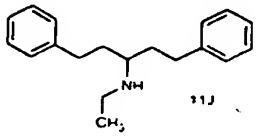
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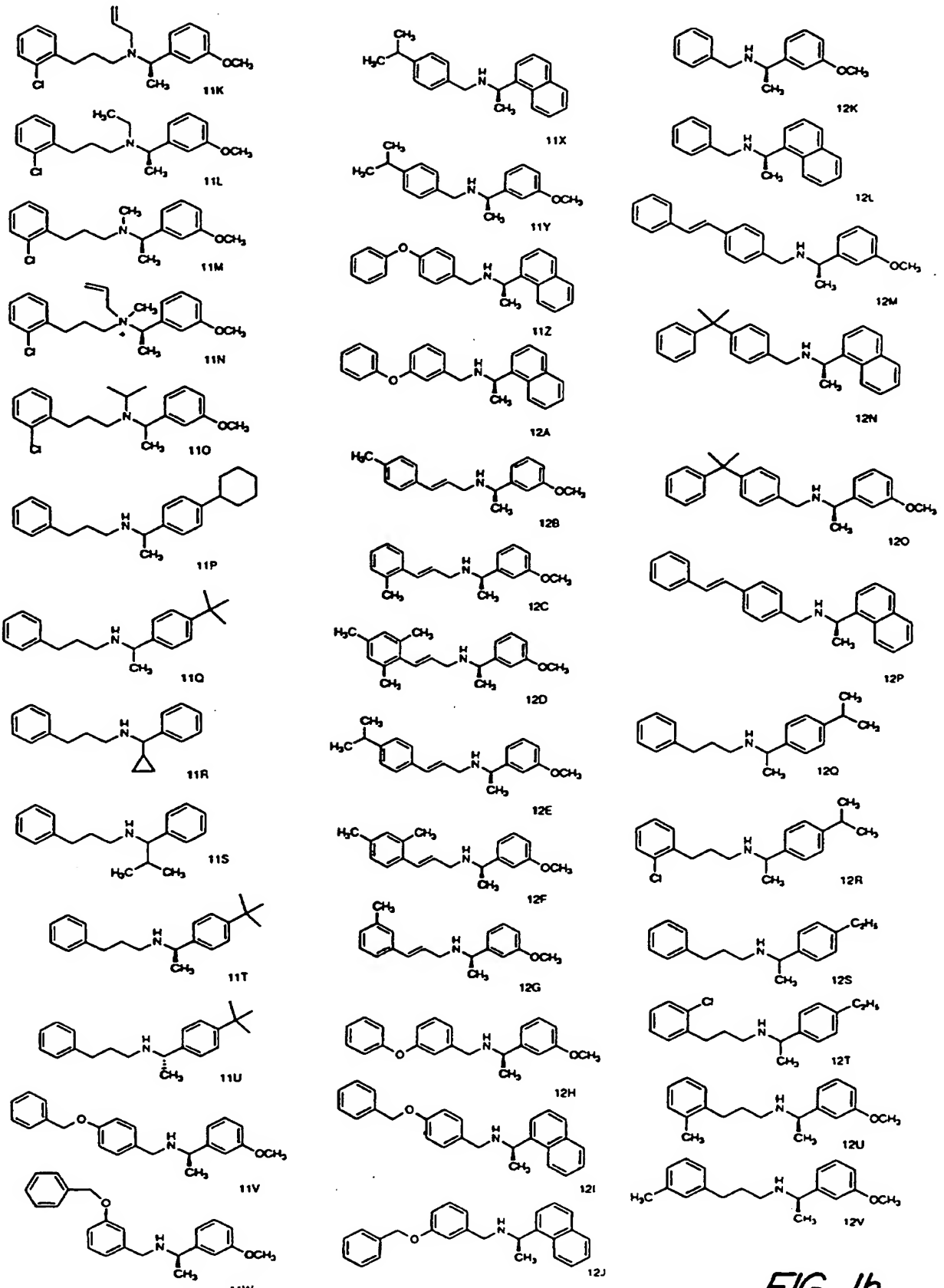
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FIG. 1h.

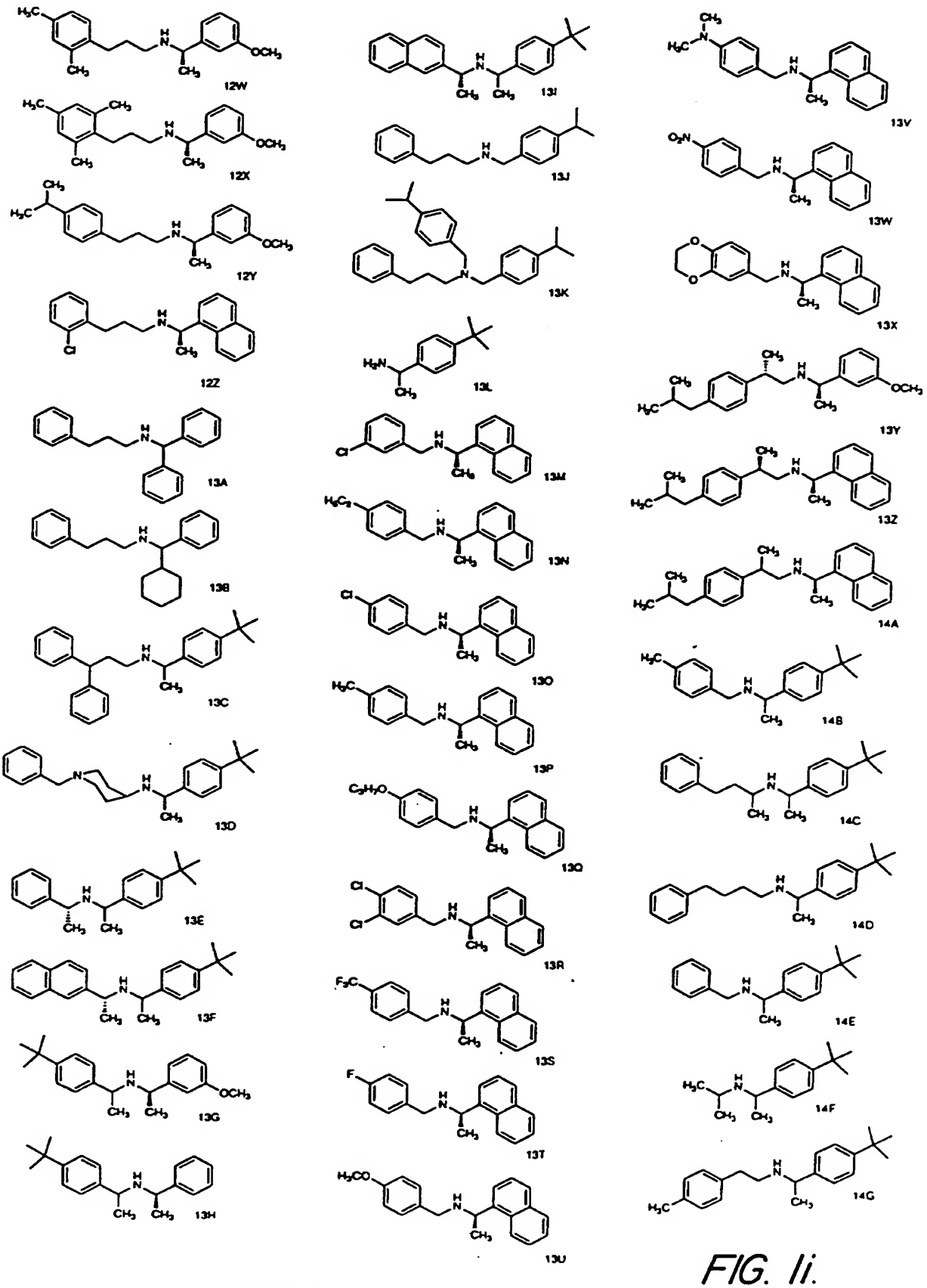
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FIG. 1i.

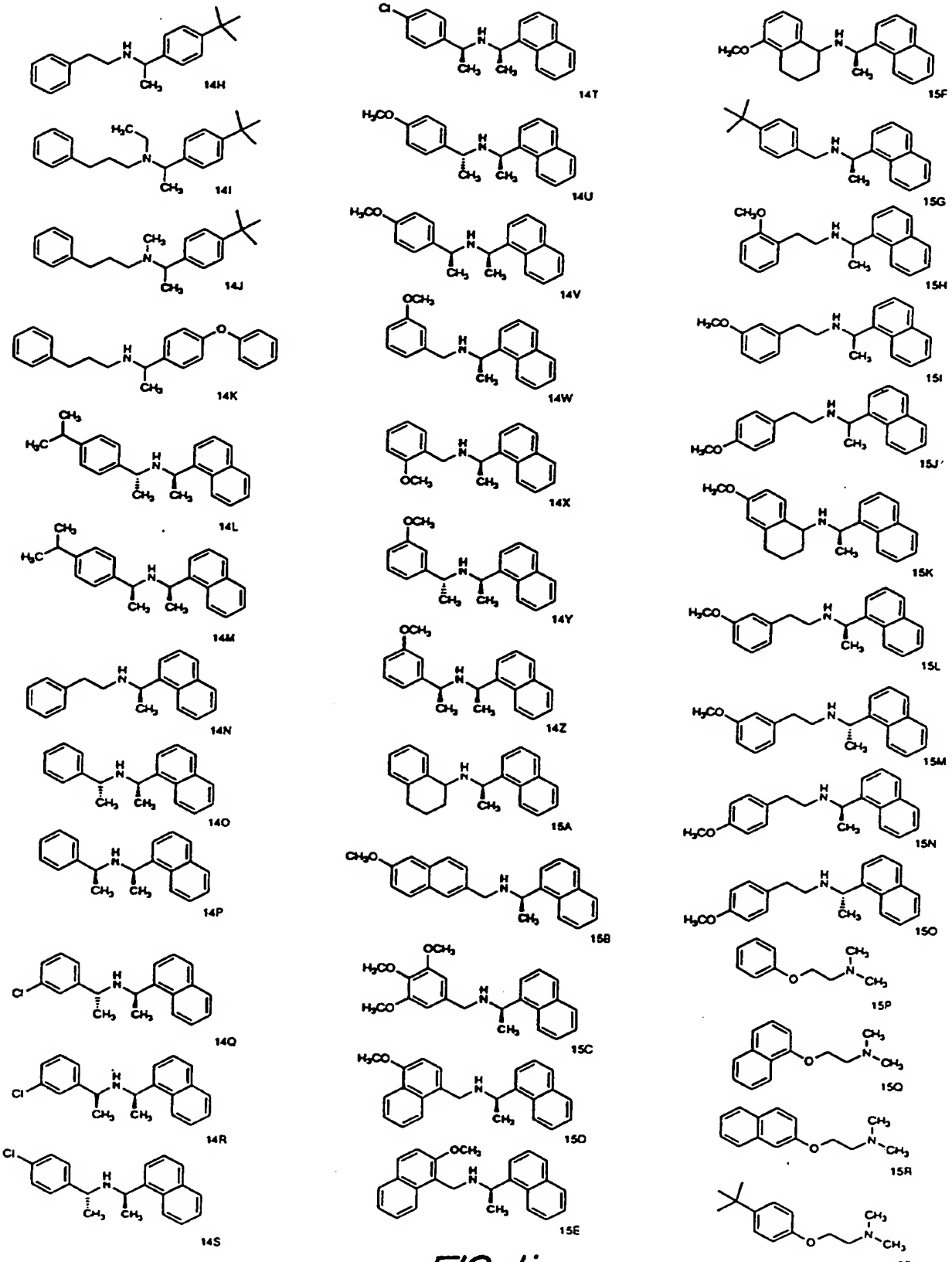
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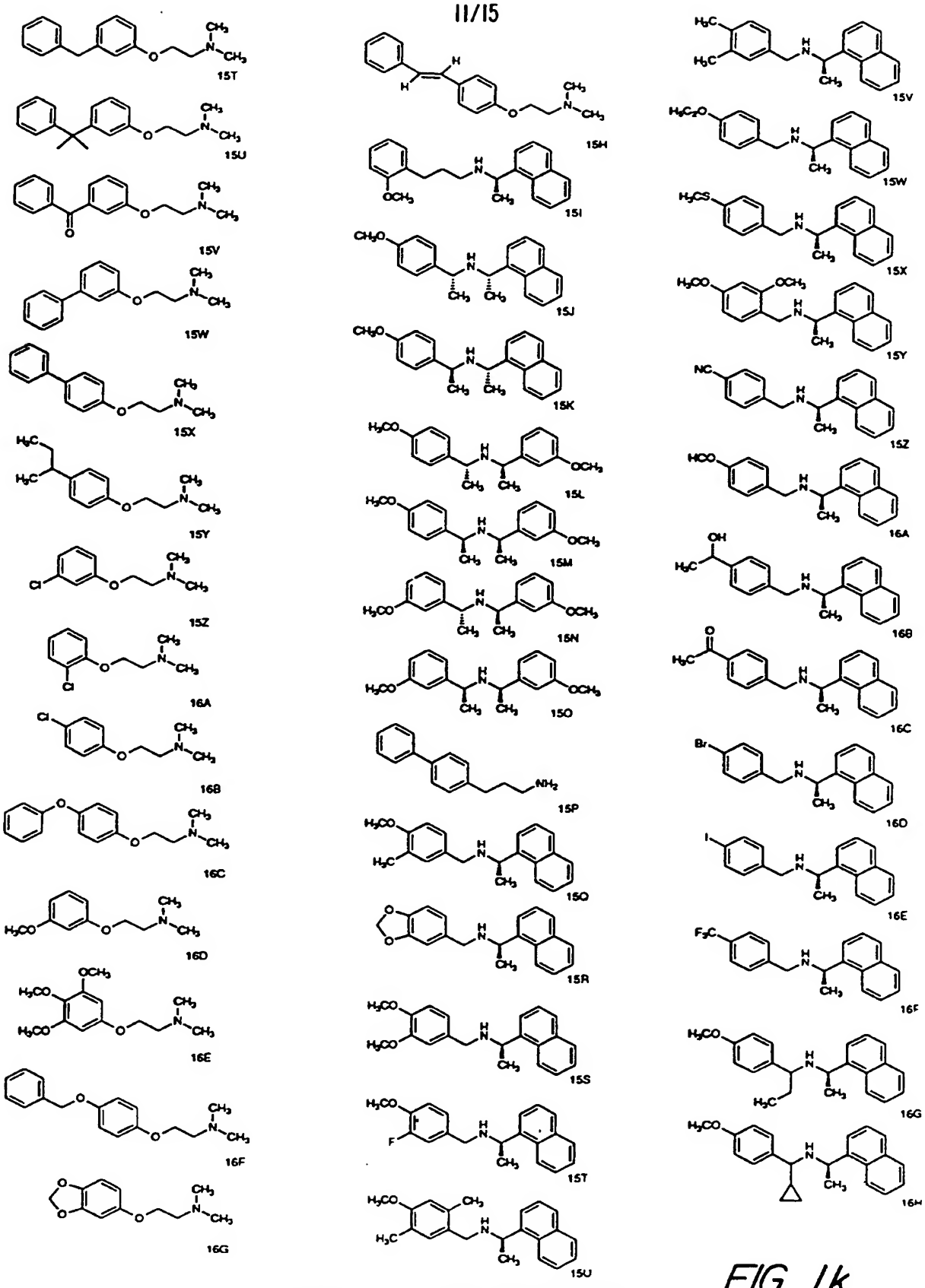
FIG. 1j.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16H

FIG. 1k.

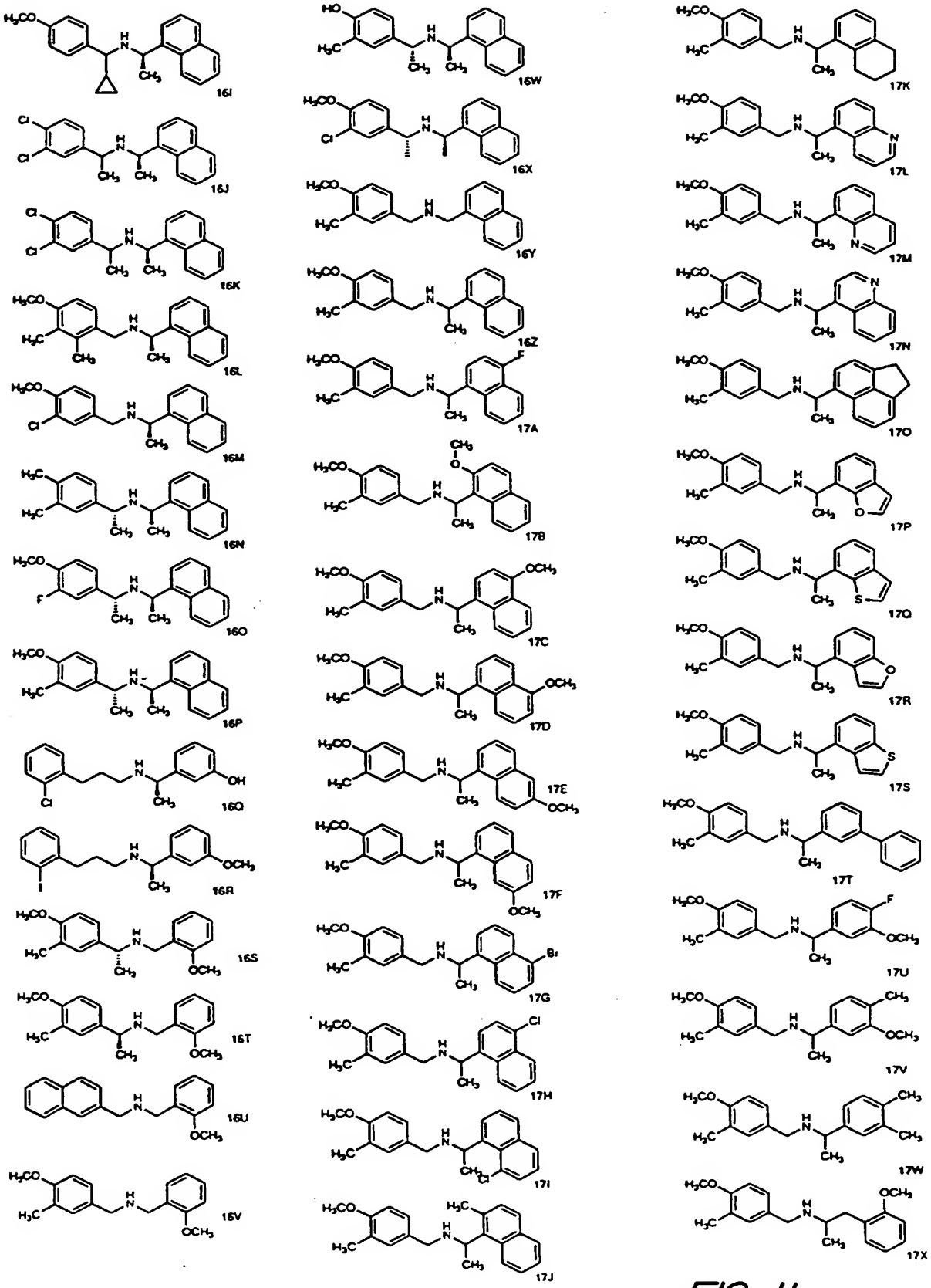
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FIG. IL.

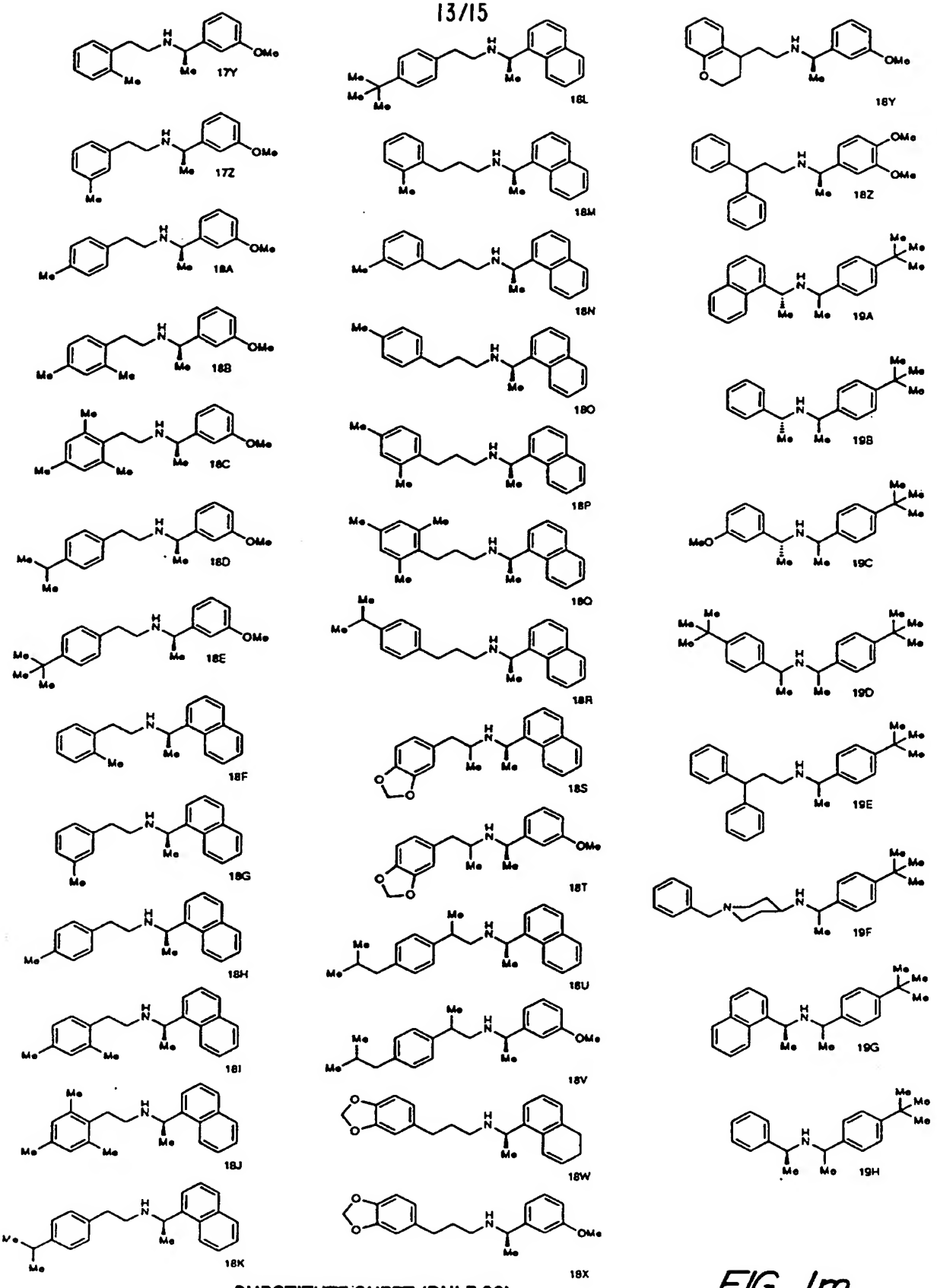
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FIG. 1m.

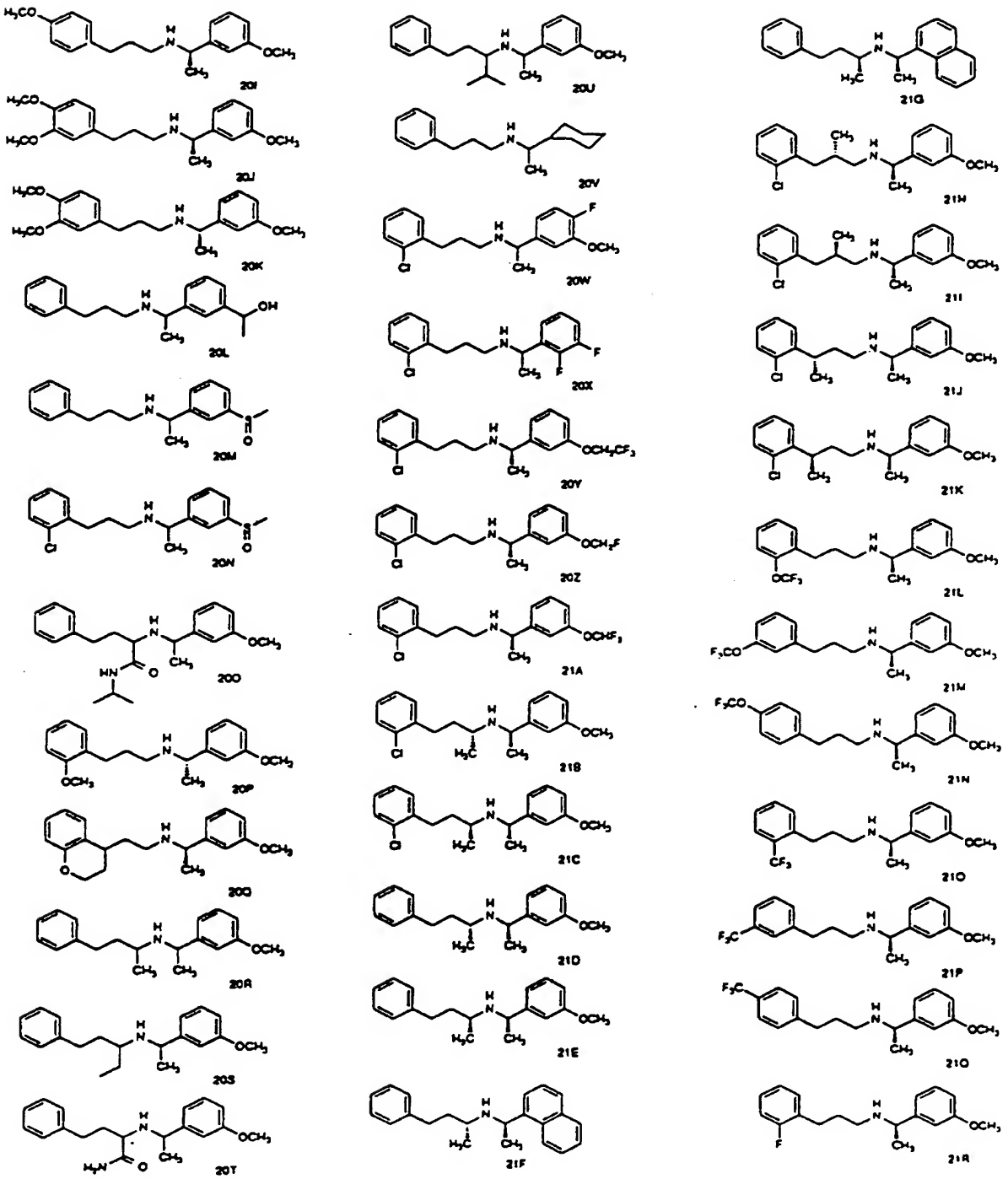
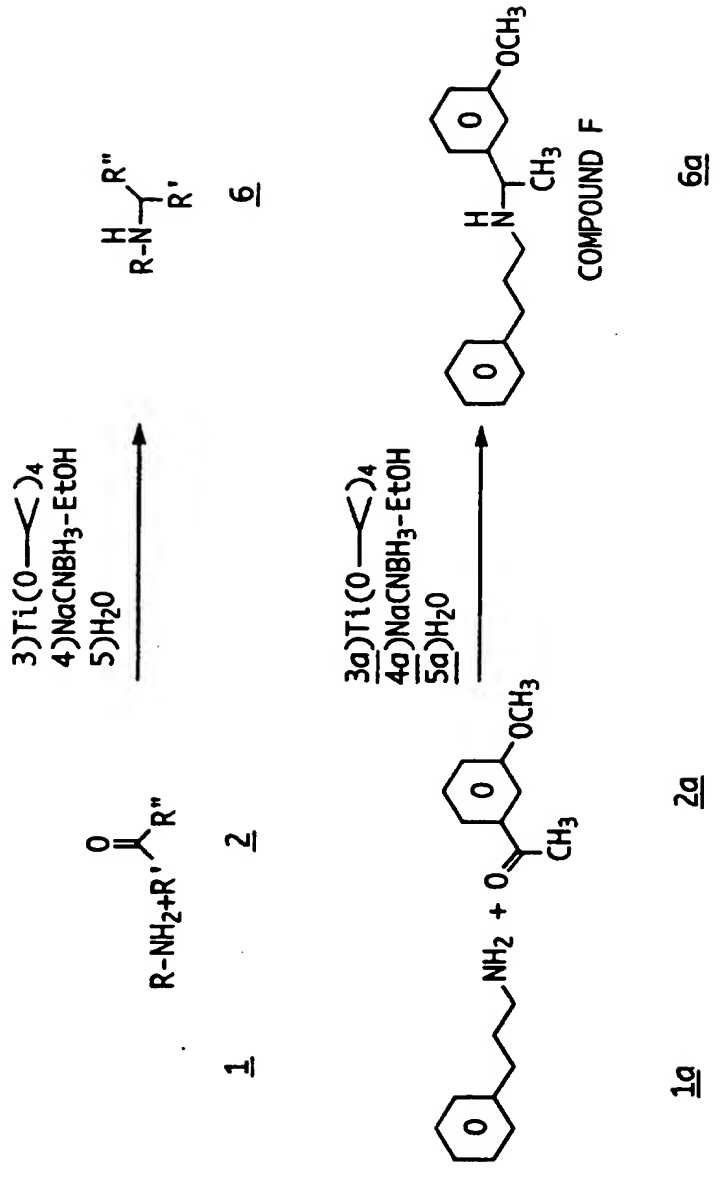


FIG. 1n.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4)NaCNBH3-EtOH
5)H2O
R-NH2+R'
R"
1
2
6
3a)Ti(O—iPr)4
4a)NaCNBH3-EtOH
5a)H2O
NH2 + O
CH3
OCH3
1a
2a
6a
COMPOUND F

FIG. 2.

INTERNATIONAL SEARCH REPORT

Int onal Application No
PCT/US 95/10745

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/135 A61K31/335 A61K31/35 A61K31/40 A61K31/415
A61K31/425 A61K31/44 A61K31/445

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,93 04373 (NPS PHARMACEUTICALS) 4 March 1993 cited in the application ---	
A	J. NEUROCHEM., vol. 61, no. 2, 1993 pages 683-689, M. PIZZI ET AL. 'Attenuation of excitatory amino acid toxicity by metabotropic glutamate receptor agonists and aniracetam in primary cultures of cerebellar granule cells.' cited in the application ---	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
5 January 1996	16.01.96

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	NEUROCHEM. INT., vol. 24, no. 5, 1994 pages 439-449, D.D. SCHOEPP 'Novel functions for subtypes of metabotropic glutamate receptors.' cited in the application	
A	NEUROREPORT, vol. 4, no. 6, 1993 pages 830-832, R.M. MANEV ET AL. 'Polyamines modulate the function of transfected glutamate receptor mGluR1A.' cited in the application	
A	NEURON, vol. 12, no. 5, 1994 pages P1121-P1129, R.W. GERAU IV ET AL. 'Potentiation of cAMP responses by metabotropic glutamate receptors depresses excitatory synaptic transmission by a kinase-independent mechanism.'	

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INTERNATIONAL SEARCH REPORT

Int tional Application No
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		NO-A-	940581	25-04-94
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